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BAYER LEVERKUSEN (GERMANY)

Department for Plant Protection

Changes in the Reaction Chains in the Insect Organism Caused by Diethyl-p-Nitrophenyl Thiophosphate

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Introduction and formulation of the problem

Opinions expressed on the site of attack, the mechanism of action and the cause of death in insects differ greatly in the very large number of publications dealing with lipophile contact insecticides. These opinions, arising out of the remarkable and striking symptoms of poisoning observed in insects, are based chiefly on the knowledge of vertebrate physiology. Further studies on the relations between the symptoms and the direct causes of them as known in respect of the vertebrata, often revealed similar chemico-physiological or organic-physiological changes in insects.

It should, however, be considered that the knowledge of vertebrate physiology cannot be applied so easily to insects which in the main have a completely different organic structure, and that even physiological-chemical processes occurring in a like manner in both animal races may be, and in fact have been proved to be of greatly varying importance for the preservation of life. Moreover, it should be borne in mind that all organs are more or less affected by a change which leads to mortality.

In order to provide an answer to the question as to the cause of death and also the site of action by the poison, it is important to establish in what order, at what rate and in what manner each pathological change occurs and spreads.

In this paper, the different changes brought about following poisoning by an insecticide belonging to the group of phosphoric esters, namely diethyl-p-nitrophenyl thiophosphate (Schradler, 1952) — also known as "Folidol-E 605" — will be traced back in stages to their causes, giving special consideration to their commencement and their progress.

Material and methods

Not every insect is likewise suitable for examining the changes which take place in the different organs or in their performance, so that naturally different species are used to deal with each particular question requiring clarification. Furthermore, the different insect species vary in their degree of susceptibility to a contact poison. The one species dies probably after only a few minutes

* Thanks to the cooperation given by Farbenfabriken Bayer Aktiengesellschaft, numerous tests were conducted in the laboratories of the Biological Institute (Director: Dr. A. Moenikes) at Leverkusen.

have elapsed while the other continues to live for several days before the poisoning finally causes its exitus. In order to be able to compare the course of the processes observed in several susceptible insects, they will be placed in relation to the externally visible poisoning symptoms which always follow each other in the same manner. It will be assumed that on the whole the same active structure is the basis for all insects. There appears to be no forcible reason to doubt the justification of this assumption.

Depending upon the problem set, the following insects were used:

Musca domestica L., 3 to 6 day old imagoes taken from a laboratory breed hundreds of generations old;

Aedes aegypti L., larvae of a several year old laboratory breed which originated from the chemotherapeutic laboratories of Farbenfabriken Bayer Aktiengesellschaft, Elberfeld (Mudrow-Reichenow, 1951);

Dendrolimus pini L., caterpillars belonging to the 8th laboratory generation of a population originating from the infestation area administered by the Forestry Authorities at Gartow, near Dannenberg, Elbe. The caterpillars develop at very different rates. In view of this, only those caterpillars were used for comparative studies between the 4th and 7th day following their last ecdysis, which — selected beforehand for equal size — had moulted within three days;

Bombyx mori L. The caterpillars were used for tests between the 2nd and the 5th day of the last caterpillar stage.

Leptinotarsa decemlineata Say. The beetles and larvae were obtained from outdoors as required, in certain cases also kept in the breeding chambers on potato leaves;

Melolontha vulgaris L. The beetles originated from infestation areas of the Aachen and Würzburg districts in 1951. They were kept at a temperature of 1° C until they were used. Only beetles showing tendency to feed, were used for the studies;

Periplaneta americana L. Imagoes taken from laboratory breeds.

The following balances were used depending upon the weight and the required degree of accuracy: a laboratory balance, sensitivity 0.001 g; a torsion balance, sensitivity of 0.0002 g; a microanalytical balance, sensitivity of 0.00001 g.

To determine the water content or the dry weight, the crushed material was briefly heated, and dried for eight hours at 110° C. This method proved to be reproducible with an adequate degree of accuracy (Hochrainer, 1924); the haemolymph and nerve plexus, however, were dried in vacuum over concentrated pure sulphuric acid to constant weight, according to the method of R. Schmaltz (Müller, 1936).

To determine the density of the blood, use was made of small pycnometers (thin capillaries curved in the form of walking-sticks) which were filled on the siphon principle. They had a volume of 1 to 5 mm³; the density was determined with doubly distilled water which was well deaerated, at 20° C. Following a little practice it is not difficult to estimate the lumen and the

capacity which the capillaries selected must have in order to fill them completely. The liquid left adhering to the outside of the short end as the result of immersion, was carefully removed under a binocular lens.

A sensitive mirror galvanometer was used to determine the concentration of hydrogen ions; the measurements were taken at 19° C.

The intensity of breathing was determined by means of a Warburg apparatus.

The oxygen formed after each period of 30 minutes at $20^{\circ} \pm 0.2^{\circ}$ C was determined volumetrically in order to examine the catalase action.

The dehydrase action was examined according to Thunberg's method (Holmberg, 1941). The tubes were evacuated for 3 minutes intermittently with a water-jet pump, the pressure in the water pipe remaining constant. The reaction itself ended at 34° C.

A colorimeter designed by Dr. B. Lange was used in combination with a mirror galvanometer for the peroxylase determination carried out according to the method of Bach and Zoubkoff.

The tissue dissected out was weighed immediately, collected in a glass tube at a temperature of up to 15° C maximum, and stored until further processed (Kiermeier, 1947). The hard-frozen tissue was reduced to small pieces, crushed and deposited in the corresponding liquids immediately before the examination was started.

In the histological examinations (Romeis, 1948), the gland tissue was fixed in mixtures according to Zenker, Bouin or Carnoy. The azan staining method according to Heidenhain gave better results than any of the many other different methods for staining the gland tissue. The nerve plexus was fixed in nonacid Formol and stained with Galloaganin according to Einarson. When the staining solution has a pH of 2.4, only the nucleus and Nissl substance are shown, whereas at a pH of 3.2 other cell parts and tissue structures also become weakly stained so that a contrast staining is not necessary.

Method of poisoning: Poisoning was carried out only with concentrated "Folidol-E 605". Any influence upon the poisonous action by solvents or diluting agents which may also have a toxic effect, was thus eliminated. A drop of poison was rubbed in a glass dish and the insects were brought into contact on all sides with the coating of poison. As a result, such an excess of poison adheres to the integument that any slight differences in the quantity of poison applied have no influence upon the course and intensity of the poisoning process. Only aqueous solutions of "Folidol-E 605" were used for insects living in water. The examinations were finished in Spring 1952.

Symptoms of poisoning

Although the symptoms observed following poisoning by "Folidol-E 605" and other insecticides have frequently been described and discussed comparatively, a brief survey will be made of the most important ones at the same time drawing attention to details which have proved to be of interest during the course of the studies.

Externally visible symptoms of poisoning

The behaviour of poisoned *Leptinotarsa decemlineata* does not differ in the early stage from that of untreated beetles; both move about in a similar manner during the latent period. The subsequent phase of excitation is sometimes preceded by a period of quiescence which may be very short and is not always noticeable. The beetles become increasingly restless, and wander backwards and forwards on the walls of the vessel in search of an escape outlet. Meanwhile the legs begin to stretch. This is first noticeable on the last pair of extremities. As if under an increased internal pressure, the last abdominal segments — normally fitting into each other like a telescope — are forced apart, and the copulation organs protrude. The male insects increase their attempts at copulation, and the female beetles deposit several eggs but not in the usual manner of oviposition. In addition to general defaecation and initial excitation, a considerable quantity of orange-red liquid (blood-like in colour) is discharged in drops out of the mouth opening of all the beetles. The mouth extremities are constantly in motion. The elytrons are raised, the wings spread out, and occasionally short attempts are made to fly. The soft-skinned tergites of the abdomen appear arched, and the intersegmental membranes stretched. Consequently the cover wings can no longer be imposed upon the abdomen in the usual manner, firmly joined together. The state of unrest finally reaches a climax, and the movements are very hasty; they appear to be uncoordinated only because of the legs being stiff. The insect, supported on its "wooden" legs, loses its balance and falls on its back. At first, it succeeds in getting up again after falling. While lying on its back, the leg movements become increasingly cramped and the intervals between each movement longer, until finally cramp-like trembling is observed, at first violent and then becoming continually weaker. Following greater excitation, defence reactions occur but these cease, too. After several hours this state gives way to complete rigidity and the abdomen collapses.

The more agile an insect is under natural conditions the more pronounced are the abnormal movements when poisoned and in a state of excitation. The house fly, for example, makes strenuous efforts in the form of movements to clean itself immediately it comes into contact with a deposit of poison. These insect's attempts to clean itself, however, are concentrated more on the tarsi — these are constantly rubbed vigorously against each other — rather than the entire body. Flies show their state of excitation by flying straight corresponding to the method of movement they prefer.

The larva of the yellow-fever mosquito, *Aedes aegypti*, normally adopts a rather vertical position of suspension on the surface of the water, and remains quiet. Occasionally it swims with a few, short, jerky strokes to the bottom, and after a time allows itself to be carried back to the surface making hardly any movement in the process. If the water contains only a trace of "Folidol-E 605", the period the larvae stay at the bottom of the water gradually shortens. The movements become hastier, longer, more violent and less relaxed, so that they appear to be less directed, and the insect finds it increasingly difficult to reach the surface of the water. Finally the larva remains motionless at the bottom or suspended to the surface by adhesion.

Symptoms are observed on the practically naked caterpillars of the silkworm — distinguished by their length and size and their scarcely sclerotized integument — following "Folidol-E 605" poisoning which cannot

be detected on rigid, armoured insects. When a droplet of "Folidol-E 605" is applied to the tergites of an abdominal segment, it is mostly caused to fall into the fold on the intersegmental membrane in front of the segment by the movements of the caterpillar. Shortly afterwards, this site and its surroundings swell slightly. The swelling then spreads segment by segment in an anal direction giving the impression that the different muscle parts contract in succession. An interesting feature is that the segments located in front of the site of application do not swell at first. Instead the swelling starts on the thoracic segments located near the head, and spreads in the direction of the abdomen. The flatulence of the thorax is not always delayed until the last abdominal segment has become swollen but depends to such a degree upon the site of poisoning that the thorax segments are caused to swell all the sooner, the closer the abdominal site of application is located to the thorax. As the swelling is a continuous and slow process, it is difficult to determine it numerically. It becomes noticeable, however, by comparing several caterpillars poisoned on different tergites, with each other and with untreated check insects. The swelling which sets in soon after the application of poison, accompanied by cell pressure, increases to the stage of excitation which is not so noticeable among the caterpillars as in the imagoes with their better developed and more clearly differentiated extremities. The excitation phase is characterized among the caterpillars by long, vigorous snake-like winding movements which become stiffer as the poisoning increases. The expectoration of large quantities of fluid during this stage is accompanied by slight reduction of the internal pressure but the caterpillar does not relax; this occurs about twelve hours later.

The fully grown caterpillars of the pine lappet moth, *Dendrolimus pini*, about 80 mm long, are much more resistant to "Folidol-E 605" poisoning than the younger members of their species and all silkworm caterpillars. Observation of swellings which develop following the application of poison, is hindered by the dense covering of long hairs. It may happen that the stage of excitation is hardly noticeable in these caterpillars, and that the poisoning process continues for several days before the caterpillar finally relaxes. Even this relaxation followed by complete decomposition of the tissue ("formation of putrid pus") does not occur in this case as it generally does among *Bombyx* caterpillars, but instead they become shorter and shorter over a period of three to four days, their willingness to react grows weaker, they shrivel and finally dry up. The shrivelling and drying-up process is similar to that which occurs under otherwise normal conditions due to complete starvation resulting from loss of substance and water during the course of several weeks.

Organic findings for poisoned insects

The most striking of the changes caused to the internal organs following poisoning by "Folidol-E 605" is the pronounced atrophy of the body-cavity fluid, a condition observed in all insects. The reduction of the haemolymph is clearly perceptible even at the commencement of the excitation stage. During the course of the poisoning process, the quantity of blood decreases so greatly that at the end of the first phase, the knock-down position for example, it is exceedingly difficult to obtain a drop of blood from *Bombyx* caterpillars which normally have such a large amount; in fact, at this stage it is absolutely impossible to obtain any blood at all from insects which usually have

little of it. The blood decrease in the relatively resistant, fully grown *Dendrolimus* caterpillars is apparently not so great as in the younger caterpillar stages or in insects more susceptible to "Folidol-E 605". A little blood can still be obtained from the former — although not always — 48 hours after poisoning, at a time when a reaction to external irritation can hardly be detected with a lens.

Whereas the quantity of body-cavity fluid decreases, the intestine fills perceptibly to such an extent that it appears thicker and more tense than in untreated insects. The contents of a silkworm intestine in this state, that is when the caterpillar has received no fresh fodder for three days, consist of a weakly coloured greenish-yellow fluid and a few fragments of fodder. In addition to the intestine, the glands of the fully grown caterpillar fill up with secretion at a rate much larger than can be observed in untreated caterpillars of the same age.

Not all the organs lose their normal turgescence at the same time; as soon as fully grown and poisoned *Dendrolimus* caterpillars, for example, become initially incapable of reaction, it is observed that the testes shrivel up in folds. During dissection under a binocular lens, it was established for all the insects examined that there was a progressive slackening of the elasticity and a decrease in the tensile strength of the nerves (from the early knock-down position onwards) and the intestine (from a later knock-down position onwards).

Comparison with poisoning symptoms caused by other insecticides and the discrimination of factors modifying the symptoms

The symptoms of poisoning are extremely similar for the numerous lipophile contact insecticides — an indisputable fact. But in view of the varying chemical constitution of these poisons it is possible that they attack at completely different points, and therefore that they cause symptoms, also during the poisoning process, which are correspondingly characteristic of the chemical constitution of the poison, and of the significance of the site of action for the vital functions.

Very detailed studies and observations have been conducted (Wiesmann, 1952; Pfaff, 1955) in order to be able to establish from the poisoning symptoms, which poison has been used. It is to be understood from these studies that the contact poisons may act more or less rapidly, in other words that the length of the latent period and the speed of the poisoning process leading to the exitus of the insect, vary at comparable dosages of poison. Specific features which indicate a different mechanism of action, are not evident, however. The eight symptoms caused by poisoning with DDT, HCH and phosphoric ester — pointed out and compared by Wiesmann — are most instructive. According to the degree of symptom development, and under the given testing conditions, these three insecticides should be placed in the following order: DDT, phosphoric ester, HCH. Even the autotomy of DDT-poisoned mosquitoes can also be attained with other preparations (Eichler, 1953; author's own observations). These differences are not qualitative but merely of a quantitative nature.

There may be numerous reasons for the variability in the discrimination of poisoning symptoms in one and the same species even when only one poison is used. The symptoms of poisoning in their entirety, the rate of the poisoning

process, the duration of the successive, poisoning phases and the discrimination of the individual poisoning symptoms, are by no means the same in every case and under all circumstances (Bauer and Gessner, 1934). This depends upon various environmental conditions (Gaines and Dean, 1949; Häflinger, 1948; Buck and Keister, 1949), differs for animals of unequal physiological value (Beard, 1949), and can be varied with the concentration (Frohberger, 1949; Lord, 1950), the solvent for the poison (Roan, Fernando and Kearns, 1950; Langenbuch, 1953), the site of application (Schaerffenberg, 1949) and the mode of application (Menusan, 1948; Metcalf and March, 1949; Langenbuch, 1952; Pfaff, 1952). In addition to the affinity of the applied poison to its point of attack, the following factors are also of decisive importance for a chemical or physical-chemical change in an organism:

- a) the quantity and concentration of the poison present at the site of action, and
- b) the ability of the organism to compensate for the change again by means of its regulatory powers.

In the case of lipophile contact insecticides, two groups of factors are of special importance for the quantity of poison acting at the point of attack: the conditions for the penetration of the poison into the body, and the measures directed by the organism against the penetrated poison before it reaches its site of action.

The quantity of penetrating poison is determined

1. by the poison's dissolving power in the epicuticle. The lipides of the epicuticle, however, are species-specific (Lees, 1947), as also illustrated by their different melting points (Wigglesworth, 1950). The dissolving power of different lipophile substances varies in one and the same solvent (Roan, Fernando and Kearns, 1950), and may even vary considerably between the isomers of one insecticide (Armstrong, Bradbury and Standen, 1951; Armstrong, Bradbury and Britton, 1952);
2. by the concentration of the applied poison;
3. by the solvent in which the poison is dissolved; it influences the solution pressure of the poison (Langenbuch, 1953; Roan et al., 1950). It should also be pointed that the solvent itself may cause a change in the epicuticle (Wigglesworth, 1941, 1944; Kalmus, 1944; Alexander et al., 1944);
4. by the number and size of the points of entry. The chief sites of penetration through the cuticle are those covered by only one epicuticle (Wiesmann, 1949; O. Böhm, 1952; Pfaff, 1952; with detailed references). The points of entry — chiefly the sites of the cuticular sense organs and the setae, perhaps also thin intersegmental membranes — are not equally aggregated at all locations (Schaerffenberg, 1949), and their number probably varies among the different species. It is, therefore, understandable why the poisoning of the tarsi with their numerous sense organs (Hayes and Liu, 1947) is particularly effective on flies (Wiesmann, 1947; Pfaff, 1952). The localization of the site of attack on the basis of the varying progress of poisoning

following application of poison to different parts of the body (Ball and Beck, 1951) is, however, not justified for the simple reason that the points of entry are not all equivalent and not equally aggregated at all locations.

There are three known possibilities, which are presented singly or also jointly, for eliminating poison which has penetrated into the body:

1. The degradation into an ineffective form. For example, DDT is broken down to DDA — dichlorodiphenyl acetic acid — or to DDE — dichlorodiphenyl ethylene — (Ferguson and Kearns, 1949; Hoskins, Perry, Fulmer and Tayhori, 1951). This may occur so quickly and thoroughly in that certain strains of *Musca domestica*, for example, are more resistant than others or completely resistant to this poison (Perry and Hoskins, 1949; Sternburg, Kearns and Bruce, 1950; Lindquist, Roth, Yates, Hoffman and Butts, 1951). "Folidol-E 605" is broken down via p-nitrophenol to p-aminophenol glucuronide, and other phosphoric esters are broken down to inorganic phosphorus compounds (Roan et al., 1950; Panhaskie, Fountaine and Dahm, 1952). The effect of the insecticides of the phosphorus compounds is in all probability closely linked with the hydrolyzability of these esters (Schradler, 1953).
2. The deposition of the poison in certain organs. DDT is stored in the fatty compounds (Lindquist et al., 1951 b), so that its influence on the site of action is withheld until the adipose tissue is broken down. An accumulation of this nature does not apply in the case of the phosphoric esters (Roan et al., 1950).
3. The poison is quickly excreted out of the body (Roan et al., 1950; Lockau and Lüdiche, 1952).

The many different factors are, therefore, opposed by the accumulation of the poison at the site of action and thus determine the extent of the changes in the organism occurring there.

The complete recovery of insects apparently affected by the poisoning and even those which are very seriously harmed, shows that not every change at the site of action has a fatal effect, and also that regulatory mechanisms intervene during the course of the poisoning process (Krüger, 1931; Schaerffenberg, 1949; Enigk, 1949; Becker, 1950; Hopp, 1953; author's own observations of *Musca domestica* and *Aedes aegypti* larvae harmed by "Folidol-E 605"). The dosage of poison which causes a state of sickness that can be overcome, is either very or not so very limited depending upon the affinity of the different poisons to their points of attack and their rate of accumulation. It is here and in the purely technical difficulty to measure the dosage correctly for small organisms like insects — this is much easier both for larger animals and for fish (Rögner-Aust, 1951) as well as for mammals (Wirth, 1949; Hecht and Wirth, 1950; etc.) — that these causes may be found in various instances when only insects remaining healthy or only fatally affected insects were observed in certain tests (Langenbuch, 1952).

The brief study of the conditions governing the accumulation of contact insecticides at the site of action has shown that numerous factors are involved

in determining the rate of the poisoning process and the discrimination of the symptoms of poisoning. Consequently, the gradual differences in symptoms, which sometimes appear to be most considerable, are made comprehensible.

In view of the externally visible symptoms of poisoning, and as poison-specific characteristics cannot be established, the question remains as to whether the lipophile contact insecticides primarily cause chemical or physical changes. The latter possibility is supported by the fact that very similar chemicals are partly extremely toxic, and partly fully ineffective — e.g. the isomers of HCH (Armstrong et al., 1951, 1952) — and that the degree of their toxicity appears to be closely linked with the solubility in the lipides of the epicuticle. It is most interesting that methods of application other than those under discussion do not bring about any fundamental change in efficacy. Just as the different chemical constitutions can be quoted for the hypothesis of the different points of attack, the lipide-solubility common to all organic contact insecticides can be quoted in respect of a physical change caused by it.

Loss of weight

One of the general symptoms of poisoning is the excretion of different substances from the body during the poisoning process. It is obvious that considerable quantities of fluid are discharged in numerous insects. The unusually vigorous movements indicate an increased consumption of oxygen, corresponding to the quantity of oxygen inhaled and the greatness of the respiratory quotients, a loss of weight is involved due to the higher specific gravity of carbon dioxide. Strong aeration of the tracheae may favour the emission of water vapour especially when the body temperature rises due to increased energy change during the excitation phase; the cuticular transpiration might also increase for this latter reason. In fact, this would probably be facilitated in that the lipophile poison changes the evaporation-inhibiting epicuticle. It should also be pointed out that in respect of other contact poisons possessing a very similar action, an increased secretion of the cutaneous glands has been established, and that this could also be the case in the "Folidol-E 605" action.

In fact the decrease in weight of silkworm caterpillars, for example, is extraordinarily great (Table 1). At 24 %, it rises to 80 times the normal loss of weight in the short period of two hours, up to the time when the insect is first knocked down.

Table 1: Loss of weight for each 10 *Bombyx mori* caterpillars.

Interval in hours	Weight of untreated caterpillars expressed in g			Loss in %	Weight of "Folidol-E 605"- treated caterpillars in g			Loss in %
0	16.412	16.775	16.560	—	16.576	16.472	16.508	—
2	16.357	16.775	16.560	0.3	12.572	12.667	12.556	23.9
5	16.308	16.704	16.495	0.6	12.118	12.254	12.002	26.6
13	16.156	16.544	16.343	1.5	11.133	11.216	10.916	32.8

Thus the body weight, a property characterizing in an elementary manner the physiological state of an organism, has changed considerably.

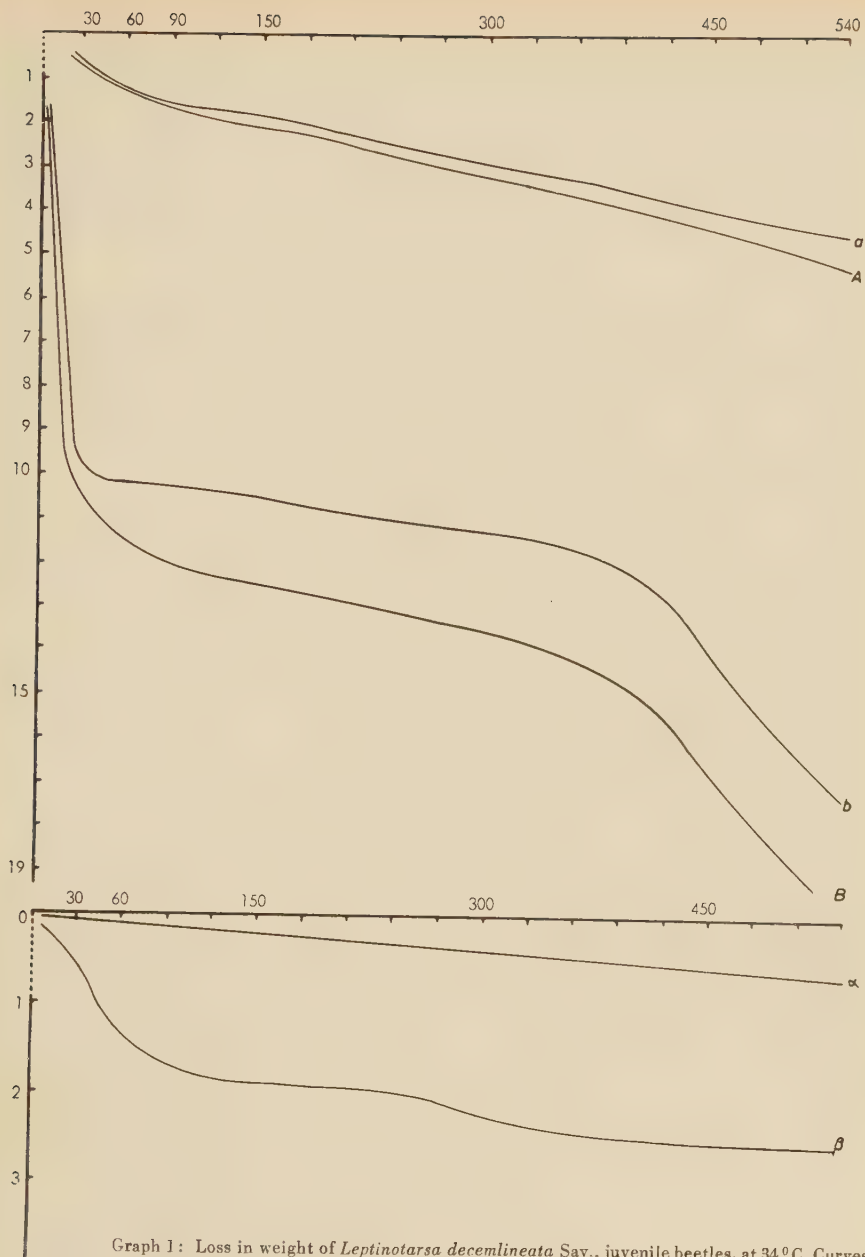
Such an appreciable and rapid decrease in weight carries with it the danger to all organisms that two important prerequisites for their existence will be destroyed, namely the necessary quantitative proportion of substances present in the body — particularly when only one or several substances are eliminated — and furthermore the likewise essential and fully determined distribution and arrangement of these substances, this all the more so the more speedily elimination takes place and the more closely limited the site of excretion. The quantitative proportion of body substances, however, varies among the insect species corresponding to their manner of feeding and habits. It even fluctuates in one individual as it develops. Therefore, it is not to be expected from the very outset that all insects lose the same percentage of weight. As already mentioned, the loss of weight for silkworm caterpillars up to the stage when they fall on their backs, is 24 %; for potato beetles — which are by no means so susceptible to poison — it is only 9 % under the same test conditions up to the same stage of poisoning; for June beetles, yellow mealworms and various Lepidoptera, the loss of weight is in fact hardly greater than usual, even though they are rather susceptible to poison. Apart from the different quantitative proportions of the individual substances, the varying loss of weight may also be attributed to the excretory organs which, in each insect species, differ in their function and construction, so that in every case the same substances are displaced and accumulated but not eliminated in the same manner.

Three essential questions arise out of all this:

1. When does the loss of weight begin and how does it proceed?
2. What is eliminated?
3. Where and how does excretion take place?

Commencement and development of weight loss was closely studied on *Leptinotarsa decemlineata* Say.

Method: Potato beetles are by no means as susceptible to poison as say silkworm caterpillars, so that the process of poisoning in the former may continue for more than one day. Moreover, the physiological condition of the beetles of one population differs somewhat considerably, which may be due to their varying age. Therefore, only young beetles were used for the following weighings, which are recognized by their fresh orange-red colour. But even they do not by any means react as uniformly as the thoroughbred silkworm caterpillars. The advantage of young beetles is that irregular oviposition is still not possible; their loss of weight can, therefore, be compared better with that of the check insects. Poisoned insects excrete their faeces only once at the beginning of the excitation phase. As the check insects also discharge their rectum within a certain period, the beetles were exposed at the start of the test to a temperature of 34° C. This temperature is well tolerated by potato beetles. Moreover, it tends to accelerate the poisoning process in treated insects. The excitation phase begins after a latent period of approximately 25 minutes, the insects fall on their backs about 70 minutes after being poisoned, and the relaxation is clearly perceptible after an interval of 330 to 360 minutes.



Graph 1: Loss in weight of *Leptinotarsa decemlineata* Say., juvenile beetles, at 34°C. Curves A, a, α are for healthy beetles, curves B, b, β beetles poisoned by "Folidol-E 605". A and B: loss in weight of beetles; a and b: weight of the excreted solid and fluid substances; α and β : difference between A and a, and between B and b. Abscissa: time expressed in minutes. Ordinate: Loss in weight expressed in percentages of the starting weight.

Batches of 15 beetles were placed in tightly closed glass dishes kept in thermostats at a temperature of 34°C . The dishes were lined with absorbent paper so that the secretion products could be kept better. They were removed from the thermostats every 30 minutes, and cooled off for three minutes at room temperature; the water vapour condensed on the walls of the vessels. The beetles were then transferred quickly to a second dish of the same type. In this way, the loss in weight of the beetles, and the weight of the excreted solid and fluid substances could be determined. For each subsequent weighing, the beetles were returned to the vessel in which they had previously been placed.

The results of three replications are plotted in Graph 1, expressed as mean values in percentages based on the starting weight of the beetles. The body weight of the check insects — curve A — decreased by 5.15 % in 540 minutes ($P = 0.0315$). The weight starts to decrease linearly ($P < 0.001$) after the test has been in progress for 60 minutes at a loss rate of 0.0053 g per 15 juvenile beetles and per 30 minutes ($P < 0.001$). During the first two half-hour periods, the average loss of weight, at 0.017 g ($P = 0.006$), is about twice as great due to the sudden rise in temperature.

The weight of the excreted solid and fluid substances — curve a — increases proportional to the drop in body weight. Here too, excretion is greater ($P < 0.001$) during the first two half-hour periods, in which the determined excretion rate of 0.0143 g is to be regarded as most exact ($P = 0.0019$), than in the whole of the subsequent period when there is a constant excretion rate of 0.0046 g ($P < 0.001$).

The difference between the loss in weight and the weight of the excreted solid and fluid substances increases strictly linearly. This difference is significant at 0.00076 g per 15 beetles and per 30 minutes ($P = 0.0072$). It indicates that a loss in weight is also brought about by the gas metabolism. This loss of weight is naturally very slight; therefore, it has been shown in curve a by a double scale of ordinate.

The weighings of the poisoned beetles provide a completely different picture. The advancement of the loss in weight — curve B — may be divided into three periods. During the second period, extending from 90 to 330 minutes after application of poison, the weight decreases linearly at a mean rate of loss amounting to 0.0048 g 15 beetles 30 minutes ($P = 0.008$). During the whole of the first period, the loss in weight is considerably greater than in the second period, and certainly does not increase linearly. The decrease in weight was more than 10 % during the first 30 minutes ($P < 0.001$). The rate at which the weight decreases is less during the second half-hour and into the third period of thirty minutes, and is definitely greater at this stage than after 90 minutes have elapsed. In the third period, the loss of weight is postmortal and consequently it is of no interest because from the 330th to 360th minute after poisoning, the abdomen starts to sag whereby the disorganisation of the organism becomes obvious.

The rate at which solid and fluid substances are excreted from poisoned beetles — curve b — remains significantly constant after the first period of thirty minutes ($P = 0.045$). During the first half-hour, it is very much greater, and the weight of the substances excreted in this short time definitely amounts to 10 % of the beetle's weight.

The difference between the loss in weight and the weight of eliminated solid and fluid substances — curve β — does not increase linearly, in contrast to the check; the rate of excretion remains constant an hour earlier than the rate of decrease in weight. The value of difference obtained after 30 minutes is doubled in the second half-hour, and during the next 30 minutes increases again by the same amount registered during the first half-hour. From the 90th minute onwards after poisoning, it is not possible to establish an increase in the difference with certainty. Although it is not precluded, it cannot be proved whether the intensity again increases slightly for a temporary period after two hours of poisoning, such as the mean values appear to indicate for the period between the 270th and the 330th minute.

A comparison of the values obtained for treated beetles and for check insects shows that a considerable loss of weight is caused when the beetles are under the influence of poison. At the beginning of the excitation stage, the beetles lost 10 % of their solid and fluid substances. Afterwards, the poisoned beetles discharge with significance ($P < 0.001$) a slightly smaller quantity of such substances than the check insects, which is a most strange condition. This slower decrease in weight may be interpreted to mean that during the period when excitation becomes greater, and while the insects are lying on their backs, less water is eliminated in vapour form at the stigmata and on the surface of the body than is generally the case under normal conditions. On the other hand, the weight of exhaled gases is extremely high particularly during the excitation phase. It is significantly greater after the first period of 30 minutes than in the check insects so that for the period of linear decrease in weight between the 90th and the 330th minute after the start of the test, the slighter elimination of substance and the increased loss of weight due to gases being exhaled combine to give an intensity of the loss of weight, which does not deviate from that of the check insects ($P = 0.016$).

The amount of water contained by the eliminated solid and fluid substances was determined by drying. 0.0059 g of dry residue is left out of 0.388 g of fluid from poisoned beetles, and for the check insects 0.00126 g of dry substance is left over from 0.1024 g of fluid. Accordingly, the percentage of dry substance amounts to only 1.5 % of the eliminated fluid for the poisoned beetles, and to 1.2 % for the untreated beetles. It may be possible that components of this dry substance, dependent upon their nature and despite their small quantity, are of importance for the continuance of the organism; this is most certainly true in respect of the eliminated water. The juvenile beetles used in this test have an average water content of 68 %. After being poisoned, they therefore lose approximately 15 % of their water content at the beginning of the excitation phase. Slikworm caterpillars with 89 per cent by weight of water lost 27 % of their water content before the end of the excitation phase. It can hardly be assumed that such high percentages of eliminated fluid are superfluous for the continued existence of the organism; it will on the contrary strive to avoid further losses of water. The slower excretion of solid and fluid substances following the initial heavy decrease in weight, may be regarded as an indication of this endeavour.

It is of particular interest to establish the nature of the excreted fluid, from which parts of the body and organs it is removed and where it is accumulated, since it can be discharged so quickly and in such quantities. It is to be expected that the atrophy of blood noticeable shortly after the application

of poison is closely linked with the elimination of fluid. On the other hand, it must be assumed in view of the unusually large filling of the mid-intestine, that corresponding quantities of fluid are shifted from the body-cavity to the mid-intestine. This fluid shifted to the mid-intestine is, however, not excreted in insects with intestinal digestion, e. g. silkworms, pine lappet moth and potato beetles. This is evident from the difference in colour between the contents of the intestine and the expectorated fluid, and after defaecation has taken place once there is no further discharge from the anus. In fact, the mid-intestine remains tightly filled until the insect dies. This leaves us with the possible explanation that in the first instance, due to the effect of the poison, the tissue under the cuticle gives off a very large quantity of water vapour so that the source of supply finally becomes exhausted, and secondly that fluid from the blood is collected and discharged in the region of the fore-intestine.

Site of fluid excretion

Ligated silkworm caterpillars were examined to establish the percentages of substances eliminated on the surface of the body including exhaled water vapour in relation to the loss of weight.

The discharge of substances out of the mouth and the anus was stopped on silkworm caterpillars by fastening a ligature immediately behind the head-capsule and in front of the anus. The caterpillars are at first somewhat restless due to the presence of the ligature. After 4 to 6 hours, probably depending upon the degree of ligation, this state of excitation subsides, and after 13 hours have elapsed, they appear to be slower in reacting than non-ligated caterpillars.

Up to 30 hours after the application of the ligature, however, they are still more susceptible to irritation by contact than caterpillars in an advanced stage of poisoning. Immediately after they had been ligated, some of the caterpillars were poisoned in the usual manner. The latent period lasts approximately 45 minutes, just as for the non-ligated caterpillars. The excitation phase, however, is by no means so pronounced, and continues for a much longer period. The ligated caterpillars first fall on their backs at a time when the non-ligated caterpillars, after being poisoned, have already relaxed, namely 13 hours after the start of the test.

There are two particularly noticeable symptoms of poisoning among the ligated caterpillars. After the poison has been effective for about one hour, at the beginning of the excitation phase, the thoracic segments are clearly swollen, and as the poisoning process continues the body movements are much stiffer than those of the non-ligated caterpillars.

When a ligated caterpillar is opened after being subjected to poisoning for two hours, there is still no apparent change in the quantity of blood. The tightly filled silk glands, however, indicate that it must have already decreased. After a further three hours, an atrophy of the blood plasma may be clearly perceived. The intestine appears to be just about as greatly filled as in non-ligated caterpillars after the same period of poisoning. At the start of the knock-down stage, there is hardly any blood present. The intestine is so seriously swollen that it forces itself out through a small cut.

The results of the weighing (Table 2) show that because of the ligation, the unusually high loss of weight following the effect of poisoning which is recorded for caterpillars not ligated, is precluded (see Table 1).

Table 2: Loss of weight for each 10 ligated silkworm caterpillars, in grammes.

Interval in hours	Non-poisoned caterpillars			Loss in %	"Folidol-E 605"-poisoned caterpillars			Loss in %
0	16.288	16.415	16.360	—	16.525	16.481	16.427	—
2	16.204	16.312	16.259	0.59	16.451	16.410	16.353	0.44
5	16.109	16.218	16.207	1.08	16.395	16.346	16.289	0.82
13	16.012	16.165	16.067	1.67	16.265	16.231	16.180	1.53

The loss of weight, therefore, in insects otherwise unhindered is to be attributed most emphatically to the discharge of fluid through the mouth. It is strange that despite greater excitation, the weight of excreted substances from poisoned insects is less than that recorded for check insects which are also ligated. This reminds one of the inhibited fluid discharge in potato beetles although the breathing is probably stronger than is normally the case.

Result: The weighings have shown that the application of "Folidol-E 605" to insects can cause a speedy discharge of large quantities of fluid through the mouth opening. This unnatural discharge of fluid ends at the beginning of the excitation phase. The quantity of discharged fluid and the short period in which it is discharged after poisoning — and moreover at a closely limited part of the body — indicate that very soon after the application of poison, substances must be displaced in an unnatural manner in the insect organism.

The results of the weighings furthermore reveal that the loss of weight due to elimination of gases also increases following poisoning. In the case of poisoned insects following the drop in weight, there is no linear relation between the weight of discharged solid and fluid substances and the weight of eliminated gases.

Histological examination of the gland tissue

The entire complex of symptoms — atrophy of the haemolymph, accumulation of fluid in the mid-intestine, strong discharge of a fluid out of the mouth, which does not originate from the mid-intestine but which is withdrawn from the blood in the region of the fore-intestine — leads to the assumption that there is an increased secretory activity both in the appendicular glands of the fore-intestine and in the mid-intestinal epithelium. It was believed that histological examinations of the glands of the digestive tract would contribute towards clarifying this point.

The salivary glands, the labial glands, the mid-intestinal epithelium and the Malpighian tubes were examined in the region of the digestive tract.

I. Salivary glands of adult *Periplaneta americana* L.

The glands were dissected out without addition of liquid.

The cell boundaries in the salivary glands of an untreated insect (Fig. 1) are clearly perceptible. The plasma has a rather uniform honeycomb structure



Fig. 1: In untreated insects

which may vary in density from cell to cell. The nuclei appear homogeneous, the chromatin is at the most slightly loosened, the centrally located nucleolus is small and hardly detectable in the dense nucleus.

After the poison has been effective for one hour (Fig. 2) — the cockroach cleans itself most energetically but still does not run around restlessly to any

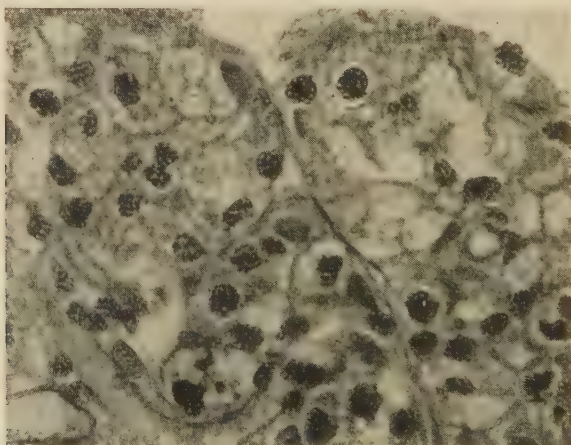


Fig. 2: After 1 hour of "Polidol-E 605" action

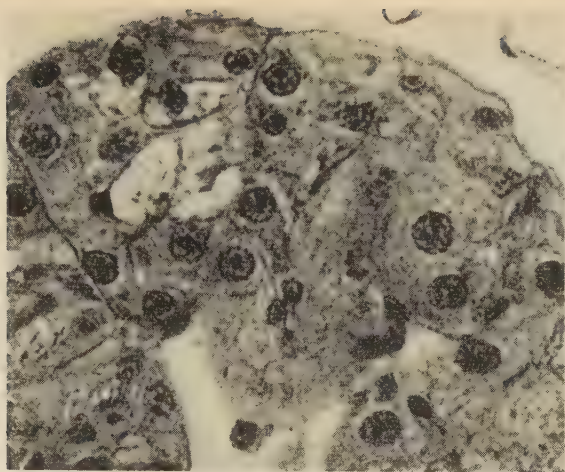


Fig. 3: After 4 hours of "Folidol-E 605" action

noteworthy degree — the salivary glands are most noticeably shrivelled. The plasma has become considerably thicker and takes up the colour much more. The cell boundaries can only be detected with difficulty, or not at all. The nuclei, on the other hand, have become larger. The chromatin in them is evenly distributed in the form of numerous grains. Chromatin bodies abut against the nucleolus, and therefore it cannot be established with certainty

Figs. 4 and 5: Labial glands of *Musca domestica* L.
Fix. Bouin 60°C; Stain: Azan; Sectional thickness 7 μ

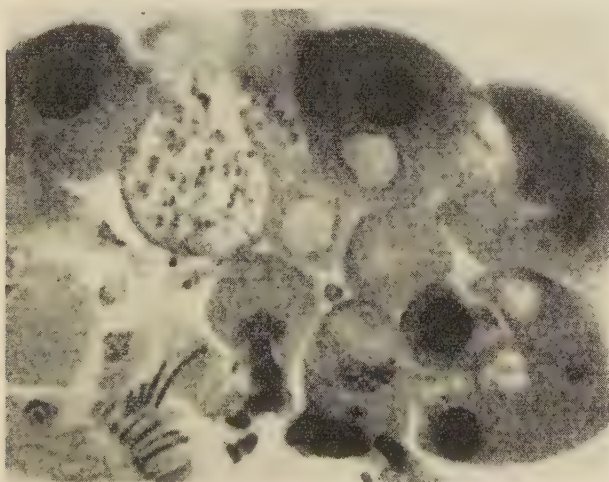


Fig. 4: In untreated flies. Magnification: 540

whether it has become bigger or not. The secretion canals are well filled. Their cells and their cell nuclei are swollen, and the chromatin is just as granular as that of the glandular cells.

After the poison has been effective for four hours (Fig. 3), the cell boundaries between the different glandular cells can no longer be detected. Large vacuoles have formed in the plasma mass. The nuclei still resist disintegration; their chromatin has become more lumpy, and the nucleolus is definitely larger than in normal nuclei.

II. Labial glands of *Musca domestica* L.

The flies were killed in hot fixing mixture according to Bouin. All the flies were dissected, and stained with Azan mixture according to Heidenheim.

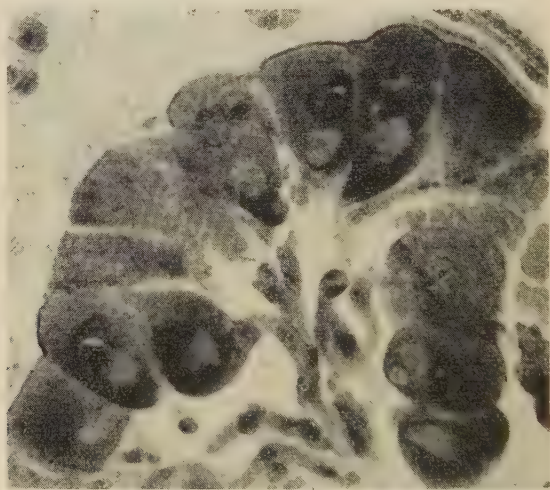


Fig. 5: Glands of "Folidol-E 605"-poisoned flies when they first lie on their back.
Magnification: 380

The plasma of the glands in the untreated flies has an almost homogeneous appearance with only a fine texture (Fig. 4). It decreases in density from the cell base towards the expulsion zone. The large nucleus with its sharp contours is densely filled with small chromatin grains. A well defined secretory vacuole can mostly be seen above the nucleus.

When flies poisoned by "Folidol-E 605" first lie on their backs, the plasma is granular (Fig. 5). The nuclei are no longer round, and the chromatin abuts against the nuclear membrane in coarse lumps. The deformed nucleolus stands out clearly against its surroundings not only because the chromatin has receded but also through its colour. It is brownish-yellow whereas the chromatin is bright red. The contents of the secretory vacuole become more deeply coloured than in cells of untreated flies, and appear denser.

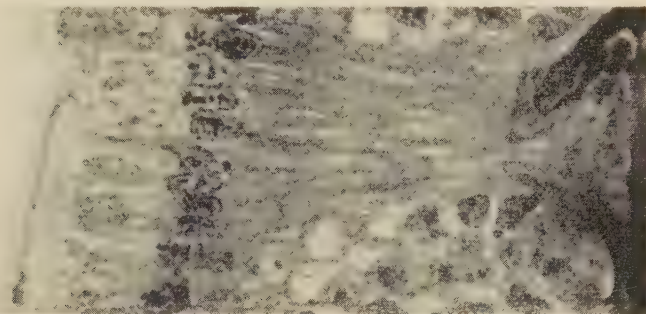


Fig. 6: In an untreated insect

III. Mid-intestinal epithelium of *Periplaneta americana* L.

Technique as described in Section I for the salivary glands.

Normally, this gland tissue consists of very many narrow and slender cells which carry a fine seam of rods on their narrow side facing the intestinal lumen (Fig. 6). The cells are divided into two halves by the location of the nucleus, namely a proximal and a distal. The plasma appears to be thickly fibrous in the basal section, and in the distal section it becomes increasingly

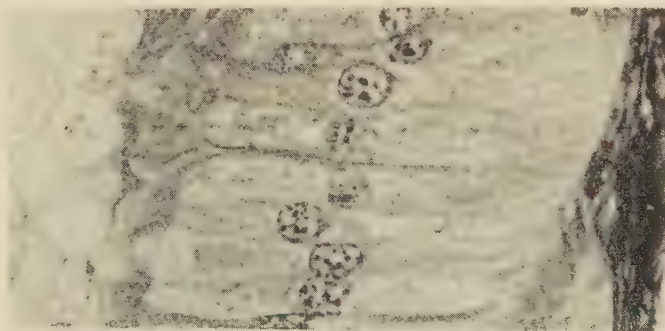


Fig. 7: After 2 hours of "Folidol-E 605" action

loose. The chromatin in the oval nuclei is granular and evenly distributed. The centrally located nucleoli are considerably larger than the chromatin grains, and are clearly perceptible.

After the poison has been effective for two hours, the whole of the plasma appears to be very loose (Fig. 7). The rhabdiorium is unevenly formed. Two large secretion sacs lie directly upon it. The nuclei have become larger and more oval, and the chromatin is more loosely distributed.

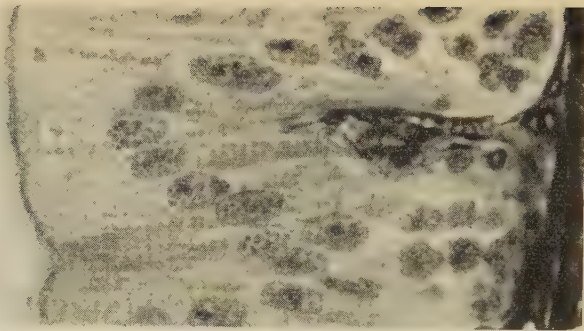


Fig. 8: After 5 hours of "Folidol-E 605" action

After the poison has been effective for five hours, the epithelium again appears almost like that in an untreated insect (Fig. 8). It is again bordered by an evenly formed rhabdorium. The cytoplasm has become thicker, but it no longer displays a ready tendency to become increasingly loose in the distal section. The nuclei are still larger than normal, and have reassumed a more flattened oval shape. The shape of the nuclei appears to be determined by the cell width. The chromatin grains are probably somewhat coarser than usual. The nucleoli, however, have definitely become bigger. Disintegration of the epithelium is not perceptible at any point.

Figs. 9—11: Malpighian tubes of fully grown caterpillars of *Dendrolimus pini* L.
 Fix.: Bouin; Stain: Azan; Sectional thickness: 8 μ ; Magnification: 430

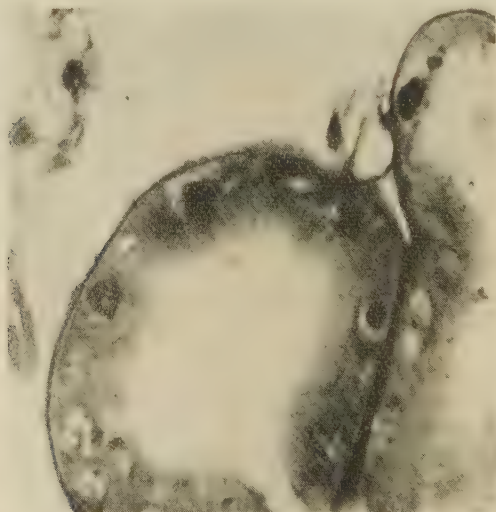


Fig. 9: In an untreated caterpillar

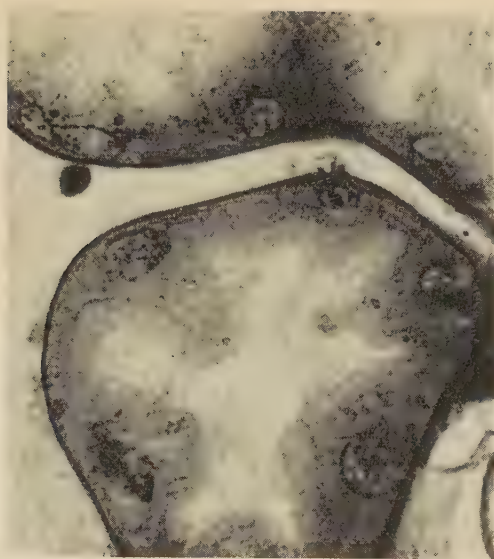


Fig. 10: After 1 hour of "Folidol-E 605" action

IV. The Malpighian tubes were taken in this case from large caterpillars of *Dendrolimus pini* L. Fixing according to Bouin, otherwise technique as above.

In untreated insects, the cytoplasm of the Malpighian tubes is thickly fibrous (Fig. 9). The height of the cells is more or less constant. The nuclei are very dense, and hardly any structures are distinguishable in them.

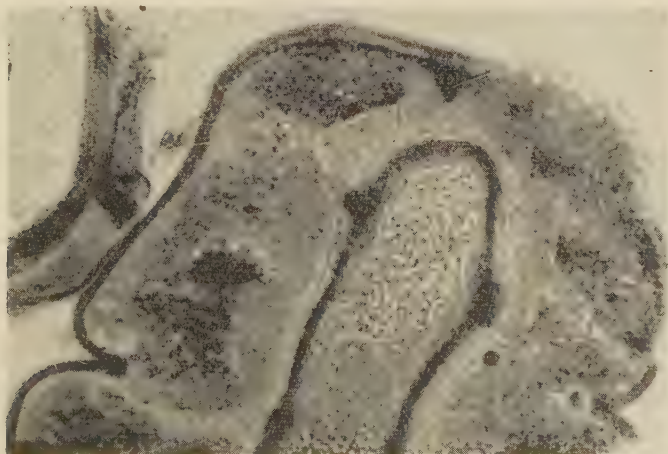


Fig. 11: After 19 hours of "Folidol-E 605" action

After the poison has been effective for one hour — the large *Dendrolimus* caterpillars are relatively resistant — the nuclei appear to be severely swelled (Fig. 10), and the chromatin is loosened. The cytoplasm projects out towards the lumen. Secretion balls are clearly perceptible in the vascular capacity.

After the poison has been effective for 19 hours — the insect still reacts well to mechanical irritation — the vascular capacity is densely filled with secretion (Fig. 11). The rhabdiorium has become narrow, and gives the impression of being a firm membrane. The nuclei have become very much bigger, and are no longer well defined. They only consist in a formless accumulation of numerous small chromatin grains.

Discussion of the gland examination

Histological changes caused by other insecticides belonging to the group of chlorinated hydrocarbons and the HCC compounds, were thoroughly examined on *Pediculus vestimenti* N. (Hopp, 1953). The findings conform to such a great extent with those obtained for "Folidol-E 605"-poisoning that both can be discussed together. Hopp tested the toxic effect of "Matacid", a mixture of ethylene trichloride, carbon tetrachloride, monochlorobenzene and p-dichlorobenzene, which is rather volatile at room temperature, also DDT and HCC in a solid and gaseous state. All secretory tissues were seriously injured by this poison. The degree of injury was determined by the concentration of the poison, its duration of action and the duration of sickness. The weakest effects were obtained with "Matacid" whereas DDT and HCC caused more serious harm. From the report, it is assumed that all three poisons act in the same manner, whereas the degree of injury caused by DDT and HCC is greater than that inflicted by "Matacid". The change caused in the labial glands may be described as follows. The secretion granules spread increasingly also in the expulsion zone, the plasma becomes granular and interspersed more and more with numerous vacuoles. The secretion which becomes thicker, remains as a compact covering on the walls of the collecting chambers. The cell nuclei of the glands, which originally are elongated, become round and bigger, and their chromatin thickens to form coarse lumps. The expulsion zone becomes denser due to the secretion granules accumulated there, whereas the formation zone becomes loosely foamy. The glandular cells themselves finally disintegrate, the cell boundaries disappear, the nuclei shrivel too, and the entire tissue becomes necrotic. In DDT and HCC-poisoned lice, disintegration sets in before the start of trembling, and at this stage the nuclei have already shrivelled up. Under the influence of "Matacid", the nuclei of the labial glands in paralyzed lice are still swelled but the cell boundaries are very indistinct.

The pictures of the labial glands of "Folidol-E 605"-poisoned *Periplaneta* with their swollen nuclei, the more compact cytoplasm interspersed with vacuoles and the disappearing and disappeared cell boundaries, may therefore be freely fitted into the pattern of structural changes as caused by chlorinated hydrocarbons.

The injuries caused by "Folidol-E 605" in the mid-intestinal epithelium of *Periplaneta* cannot be compared so well with those caused by chlorinated hydrocarbons in *Pediculus*, on the basis of Hopp's descriptions for "Matacid"-poisoning. Hopp has directed his attention chiefly to the process of mitoses; they are caused by "Matacid" and its components

but cease again in the prophase or at the latest in the metaphase. The size of the nucleus increases along its longitudinal axis, the chromatin becomes lumpy, the cytoplasm is interspersed with numerous vacuoles and disintegrates so that the brown contents of the intestine are discharged into the body-cavity. The resorbing cells which, in contrast to *Periplaneta*, can be easily distinguished from the secretory cells in *Pediculus*, are destroyed more rapidly than the latter. The intensity of secretion increases following DDT and HCC action, is no longer apocrine but holocrine, and the remainder of the plasm disintegrates. There are no more nuclei to be found in the epithelium in the paralysis stage.

The changes in the mid-intestinal epithelium as described by Hopp conform in respect of two important points with those established for *Periplaneta* poisoned by "Folidol-E 605", namely the increased secretion and the enlargement of the nuclei. A disintegration of the epithelium was not established in *Periplaneta* even after it had been lying on its back for two hours.

The aspects of poisoning in the Malpighian tubes are also more or less the same for both "Folidol-E 605" and chlorinated hydrocarbons. In the case of *Pediculus*, excretion is greater, the cell nucleus becomes bigger, the cytoplasm is loose and filled with excretion bodies in the distal section; finally the nucleus shrinks and is expelled together with the excretion-containing plasm. An important discovery is that HCC causes a much slower change in the Malpighian tubes than DDT because HCC is more effective than DDT for the secretion of the mid-intestine. "Folidol-E 605", on the other hand, causes a weaker secretion than DDT in the mid-intestine but has a stronger action than the latter on the labial glands. Accordingly, there is a certain correlation between the rates at which the labial glands, the mid-intestinal epithelium and the Malpighian tubes secrete under the influence of these poisons, in that the mid-intestinal epithelium only secretes what it contains in view of the greater activity of the labial glands, and the Malpighian tubes only excrete as much as they are able in face of the rapidly increasing shortage of fluid due to the stronger action of the other two glands. The absence of substrate required for the formation of secretion may be regarded as the direct cause of the disintegration of the gland tissue. The preferred role occupied by the labial glands in poisoned insects may be explained by the fact that within the scope of natural performances, they are capable in accordance with their task, to react extremely vigorously for a short period to corresponding irritations. The large surface area of these pseudoacinous glands enables them to extract rapidly large quantities of fluid from the haemolymph flowing around them. It now becomes understandable why it should be in the fore-intestine that the poison becomes concentrated so rapidly, whereas its concentration increases much more slowly in the mid-intestine and hind-intestine. Roan et al. (1950), who established with radioactive TEPP the tendency of this phosphoric ester to concentrate in the fore-intestine of *Periplaneta* and found radioactive material also in the tissue of the gizzard, concluded from their discoveries that the gizzard epithelium takes up the poison actively and secretes rapidly into the gizzard lumen. Ball and Beck (1951) arrived at the same results and conclusions in respect of "Folidol-E 605", basing their findings on a paper of Abbott (1926), which states that the gizzard tissue is permeable to fats. Lockau and Lüdicke (1952) who also worked with radioactive "Folidol-E 605", present the same arguments. The strongly disputed

question as to whether fats taken up with food penetrate into the body tissue in the region of the fore-intestine, was settled by Abbott by means of an experiment, in that the olive oil he fed was actually able to penetrate the chitinous intima of the gizzard in *Periplaneta australiasiae* Fab., and that it was traced in the epithelial cells of those parts of the gizzard, which came into contact with nutrient fat. The other important question as to whether, in the opposite direction, body-specific fats penetrate or may be able to penetrate through the chitinous intima into the fore-intestine via the epithelium is, however, by no means settled by this experiment. In fact, this was not discussed by Abbott. As the epithelial cells located under the chitinous intima of the fore-intestine, do not constitute gland tissue, either, it may be assumed with certainty that the poisons reach the fore-intestine from the mouth-cavity together with the secretion of the appendicular glands, and — in the sense put forward by Abbott — diffuse from the fore-intestine also into the neighbouring epithelium.

Swelling of the cells, the nucleus and the nucleolus, shrinkage of the plasm and its interspersation with vacuoles, formation of chromatin into lumps, and disintegration of the nucleus are symptoms which are continuously encountered in most cases when the cells are subjected to excessive strain, when sickness is caused and when organisms are under the influence of poison. Wermel and Ignatiewa (1933, 1934) observed these changes in different tissue cells of frogs and rats following sickness caused by "Trypanblau", "Novarsenol", "Salvarsan", "Levisit" and "Yperit". They believe that in a state of sickness, the general coordinating links in the organism are attacked; the enlargement of the cells and the nuclei is a tendency naturally inherent in the cells, which cannot spread at random due to the interaction of the many different tissues to form an organism. According to Lepeschkin (1957), coagulations, solidification of plasma and cell shrinkage represent progressive continuation of preceding swelling of cells. This swelling of the cells is brought about by greater water uptake due to the permeability of the cell bodies being increased. It is, however, a known fact that a greater water content of the cells is accompanied by a higher rate of metabolism. This is made particularly manifest by an increased uptake of oxygen (Schlieper, 1930, 1936, 1942; Harnisch, 1929, 1930; Pieh, 1936). It has often been demonstrated that the nuclei play a decisive role in metabolism (Caspersson, 1941; Landström, Caspersson and Wohlfahrt, 1941). It is, therefore, comprehensible that very active cells like the glandular cells, in particular, have relatively large nuclei. The size of such nuclei is, however, due first and foremost to an increased stock of chromatin (Risler, 1950; Olzewski, 1947). Moreover, the volume of the nucleus may also vary proportional to the quantity of nuclear fluid (Geitler, 1938a, 1938b, 1940, 1941; Risler, 1950). Wermel and Ignatiewa (1933, 1934) observed both forms of nuclear hypertrophy caused by poisons; firstly, swelling due to a greater content of nuclear fluid whereby the chromatin accumulates in drops (this swelling is reversible); secondly, rhythmic growth due to an increase of chromatin, which mostly follows amitotic nuclear fragmentation. In this connection, a toxic effect of "Matacid" and its components described by Hopp (1953) is of particular interest. These poisons are said to stimulate mitoses in embryonic tissues, but these cease at the latest in the metaphase; the chromosomes may swell, and form a resting nucleus again due to fusion. When a stronger dosage

of poison is administered, the mitosis is followed by a pyknosis. In fact mitoses were caused by "Matacid" in the association neurones which normally are not capable of segmentation. After a recovery period of 24 hours, the chromatin which due to the action of "Matacid" had formed into lumps, again had a reticular structure, and there were said to be more "chromocentres" than before. The volumes of the nuclei of association neurones increase after being exposed to the effect of "Matacid" or any of its components. The swellings, however, differ in extent. The various sizes of the nuclear volumes unfortunately cannot be classified in a regular series in the table provided by Hopp. Should the nuclear changes be correctly interpreted here as mitoses, then we would have a remarkable example to show that perennial nerve-cells have not lost their ability to effect mitotic division. Similar observations were not made in *Bombyx* caterpillars following "Folidol-E 605" action although a component and decomposition product of "Folidol-E 605", p-nitrophenol, is known to be a mitosis poison (Hindmarsh, 1952).

Many observations have been made, particularly on nerve and secretory cells, which show that there is an intimate relation between the nucleolar volume and cellular metabolism. Leshner (1951) reports on the salivary glands of *Drosophila* that the nuclear volume increases during larval development, being in direct relation to the degree of polyteny; the volume of the nucleolus, which attains its maximum before the nucleus, depends upon the secretory activity of the cell. A close correlation between the nucleolar volume and the functional phase of the cell was also established by I. Fischer (1935) in the follicular epithelium of *Linognathus piliferus* and *Pediculus vestimenti*. Here too, the volume of the nucleolus increased well ahead of that of the nucleus at the beginning of the functional phase. Bretschneider and Raven (1951) report on a substantial participation of the nucleolus in oögenesis. In *Limnaea stagnalis* L., nucleolar substances are formed both in the interior and on the surface of the nucleolus. During the formation of secretion in the mid-intestinal gland of *Astacus leptodactylus*, W. Jacobs (1928) observed several aspects which are to be interpreted as expulsion of nucleolar substance. A bright zone and vacuole form immediately around this expelled substance, in which the nucleolar substance undergoes a transformation and quickly resembles the cytoplasm in colour. The small, round and somewhat more strongly coloured structures in the vacuoles of the labial glands of *M. domestica* are also to be regarded as such "parasomes" (Figs. 4, 6). Scholtyseck (1954) reports that in *Eimeria maxima*, whole karyosomes which are newly formed several times, pass into the cytoplasm and break up there. An interesting discovery is that of Deufel (1954) who established that in *Urtica dioica* the nucleolar volume at first increases as the glandular cells develop, but then decreases again immediately prior to the formation of secretion. Detailed information is given by Caspersson (1941) and Landström, Caspersson and Wohlfahrt (1941) on the material nature of the nucleus, nucleolus and cytoplasm, and on their interrelations. A comprehensive survey is given by Stich (1956).

Hardly anything is known of changes in the nucleolar volume in sick insects, corresponding somewhat to the volume increase of the cells and nuclei frequently observed in such cases. Wermel and Ignatiewa (1933) mention that the nucleoli in the hepatic and renal epithelial cells of frogs and rats undergo strong hypertrophy under the influence of several poisons.

C. and O. Vogt (1946) discovered greatly enlarged nucleoli in human ganglion cells as the result of nerve diseases. Olzewski (1947) reports that larger nucleolar quantities are formed as a result of nerve diseases; when the supply of development material stops, the nucleoli shrink again. Lüers et al. (1953, 1954) report that following poisoning by "Folidol-E 695", the nucleoli in the ganglion cells of *Drosophila* and *Musca* are frequently peripheral, but they are not able to make any statement on changes in volume. Considering that the nerve cells of insects are very small anyway, it is only with great difficulty that measurements can be satisfactorily carried out on the nucleoli. Hopp makes no mention whatsoever of the nucleoli even in respect of the larger glandular cells.

Result: Following poisoning by "Folidol-E 605", there is every indication of an abnormal cell activity in the gland tissues. This is made manifest particularly by a swelling of the cells, the cell nuclei and the nucleoli. The increase in the volume of the glandular cells is followed by an interspersion of the cytoplasm with vacuoles and a shrinkage of the cytoplasm. The histological findings indicate that even before the start of the excitation phase, fluid is shifted from the body-cavity into the digestive tract, particularly through the salivary glands. The nature and the quantity of fluid secreted by the different insects following poisoning by "Folidol-E 605", are determined by the structure and the function of the *Valvula cardiaca*, and by the quantity of blood in the insects.

Following poisoning by other lipophile contact insecticides, the changes discovered in the gland tissues are the same as those caused by "Folidol-E 605".

Oxygen consumption in *Leptinotarsa decemlineata* Say. following poisoning by "Folidol-E 605" at 34° C

The weight measurements for potato beetles have shown that under the influence of poison, the loss of weight increases considerably due to the elimination of gases in addition to the secretion of fluid. It would seem obvious to attribute this loss of weight to a higher rate of breathing. Normally this is also accompanied by an increased elimination of water in the form of vapour. But such an increased loss of water could not be established either for potato beetles or for silkworm caterpillars; on the contrary, it is reduced. On the other hand, the state of very great excitation supports the belief that there is increased metabolism to replenish the energy deposit from reserve substances, and that consequently there is a corresponding requirement of oxygen.

Lord (1950) established the existence of an effect causing a higher rate of breathing following poisoning by "Folidol-E 605" in *Tribolium castaneum* Hbst. He was able to show that the commencement and the progress of a change in breathing depend upon the concentration of the applied poison, and that in respect of *Tribolium* a higher rate of breathing can no longer be attained following applications of concentrations stronger than 0.5 % at a temperature of 25° C. Regarding the temporal correlation of a changed rate of oxygen uptake to other symptoms during the course of the poisoning process, he merely mentioned that the increase of the O₂ rates is accompanied by a stoppage of the movements. For the purpose of our studies, more details are required on this point, and it would be of interest to establish whether there



Graph 2: Rate of oxygen uptake for healthy potato beetles — bottom curve — and potato beetles poisoned by "Folidol-E 603" — top curve — at 34° C. Abscissa: Time in minutes after application of poison. Ordinate: Quantity of oxygen in cc's taken up by 100 g beetle per minute.

are any relations between the change in breathing and the loss in weight with its components, and also the externally visible symptoms of poisoning.

Method: Juvenile beetles of *Leptinotarsa decemlineata* were again used as test insects, just as in the exact study of the weight loss. Here too, the consumption of oxygen was determined at 34° C with the Warburg apparatus. It had proved advisable in preliminary tests to work with single beetles and to enclose them loosely in a muslin cloth to prevent them from coming into contact with the potash solution. A caustification may lead to a tenfold or greater increase in the oxygen consumption, only to sink rapidly to zero; with slighter caustification, the O₂ consumption is correspondingly less, but it lasts longer. After being poisoned in the usual manner, the treated beetles were immediately placed in the Warburg flask. Readings were taken every 5 minutes.

The results are plotted in Graph 2. In the check insects, the rate of O₂ uptake with slight fluctuations caused by a sudden change of temperature, is adjusted to the higher rate of metabolism at 34° C, with an O₂ uptake of 1.5 mm³ per beetle and per minute. The mean value of 1.8 mm³ per beetle and per minute may be regarded as reliable and constant for the entire period of the test. Similar values were obtained in metabolism studies conducted on potato beetles in a state of quiescence (Marzusch, 1952).

In poisoned beetles, the O₂ rate is considerably greater at all times — also during the first 10 minutes — than that established for the checks. It increases linearly ($P < 0.01$) up to the 90th minute after poisoning, and is raised 0.8-fold every ten minutes. During the following 100 minutes, the intensity of O₂ uptake decreases again, and the O₂ rates drop more and more slowly. Once the period of maximum O₂ uptake has been exceeded, an O₂ rate is attained 190 minutes after poisoning, which does not sink again during the following period of 90 minutes. In fact it rises again slightly up to the 240th minute after poisoning, and then sinks again from the 250th minute onwards.

The change in the intensity of weight loss following the elimination of gaseous substances (Graph 1, curve β), may in fact be interpreted as being similar to the change in the intensity of respiration following an application of poison. In both cases, there is a progressive increase up to the 90th minute following poisoning, which gradually sinks only to increase again slightly for a temporary period from the 240th minute after the start of the test.

Discussion of the results: There is an increased uptake of oxygen during the first ten minutes. This shows that it can be caused not only by the motor hyperactivity. The metabolism and, consequently, also the oxygen required for this process not only serve to replenish the different energy deposits but also to produce water and to maintain the water balance, particularly in insects. It is the water of metabolism which gives xerophile species the possibility to exist on dry fodder. If the water loss is stimulated, for example by injuries to the epicuticle by different kinds of dust (Lord, 1950) or even by only a dry atmosphere (Buck and Keister, 1949), respiration increases after a certain period and consequently there is an increased production of water. The greater requirement of O₂ before the beginning of the excitation phase, therefore, arises out of the already existing deficit of water. As the intensity of respiration increases linearly from the beginning of the poisoning process onwards, and is not increased to a higher degree during the excitation phase, it is unlikely that the energy consumption for the motor hyperactivity influences the increased metabolism. In fact it is probably just

the reverse. In the process of metabolism serving to remedy the deficit of water, substances required for the muscle activity are also formed in superabundance. Moreover, the heat set free in this process facilitates a speedier reaction to normal nervous irritation. At the beginning of the excitation phase, the rate of metabolism is approximately twice as high as that in check insects. If the metabolism is stimulated in a corresponding manner in untreated beetles by raising the temperature, then these beetles will be just as excited at 45°C as poisoned beetles at 34°C . This is a further argument in support of the assertion that the motor hyperactivity is first rendered possible by the preceding increase of metabolism to balance the deficit of water. The intensity of respiration is also increased again when the vigorous movements subside. At a value averaging four to five times the normal O_2 rate, it attains a maximum at a time when the movements have apparently stopped. The second, slight increase of the O_2 rate is evidently the first indication that the tissue has started to disintegrate and is not due to an active vital function because immediately afterwards the muscles relax, and the fluid accumulated in the intestine flows out.

The change in the uptake of oxygen established in potato beetles poisoned by "Folidol-E 605" corresponds even in detail with the findings obtained for *Tribolium castaneum* Hbst. (Lord, 1950). The O_2 rate increases four to fivefold linearly for both beetle species, whereas the movements cease; subsequently the slow decrease of the O_2 rate is also stopped temporarily. The time which elapses before there is a change in the uptake of oxygen is considerably longer for the red flour beetle than for the potato beetle. This may be due to morphological and physiological characteristics of this beetle, and it is definitely caused as well by the temperature difference of 9°C . A higher temperature probably assists the penetration of poison in *Tribolium*, too, and quickly brings about the intensification of respiration.

The finding that it is not the motor hyperactivity but a deficit of water which causes the increases of metabolism, and that in remedying this condition the metabolism helps, on the one hand, to increase the body temperature due to the release of heat energy thus favouring as well the willingness of the organism to react, and on the other hand provides the energy sources with an abundance of substances for the muscle activity, appears at first to be really surprising. This mechanism, however, is probably widespread in insects; it would explain the biological significance, in particular, of the many different spontaneous expulsions of fluid accompanying any form of unrest indicating danger — reflex spitting, discharge of the coxal glands, reflex bleeding. Due to the consequences of the relatively high loss of water thus caused, the insects are enabled to make rapid movements and to take vigorous defensive action.

The extraordinarily great decrease in the quantity of blood plasma in insects poisoned by "Folidol-E 605" indicates the advisability of carrying out a close examination of the blood.

Change in the water content and in the specific gravity of the blood following poisoning by "Folidol-E 605"

Due to the strong gland activity in poisoned animals, neither pure haemolymph nor pure water is extracted from the body, and therefore the composition of the blood must undergo a change with respect to the water content

and the dry substance. This assertion was checked by testing the blood of healthy *Dendrolimus* caterpillars and others poisoned by "Folidol-E 605". The latter were in the early stage of knockdown. The following result was obtained.

Specific gravity and water content of blood in healthy and "Folidol-E 605"-poisoned caterpillars of *Dendrolimus pini* L.

	Specific gravity of blood	Water content in per cent by weight	Specific gravity of dry residue
Healthy caterpillars	1.010 [4 ± 0.00051; n = 10	88.29	1.09 [6
Poisoned caterpillars	1.021 [7 ± 0.00055; n = 10	56.41	1.05 [1

The above data show that the water content of the blood is reduced considerably due to the effect of poison. The specific gravity of the blood increases slightly. This increase is not due solely to the loss of water; namely, a decrease in the water content to 78 % would suffice to raise the specific gravity of the blood to 1.02. Together with the water, substances dissolved in it are extracted from the blood. Consequently the specific gravity of the dry substance of the blood also changes.

The change in the blood of poisoned *Dendrolimus* caterpillars is without doubt, to be attributed largely to the extraction of substance by the gland activity. There is, however, also the possibility that substances are supplied to the blood. Normally this happens after digestion. The histological picture of the mid-intestinal epithelium does not give any cause to doubt that substances are supplied to the haemolymph in poisoned animals. Consideration must be given, however, to an extraordinary discharge of substance from the different tissues whose cell permeability might be changed either by direct action of the poison or as a result of the thickened and changed state of the blood circulating around them. In this respect, attention should be drawn to the disintegration of the gland tissue, the shrinkage of the testes, the reduction of the elasticity and tensile strength of the stomach ganglion chain, in other words symptoms of disintegration perceptible before all signs of life come to an end.

Change in the water content of the nerve plexus due to the action of "Folidol-E 605" on the organism

A study will be made in this section to establish whether the water content of the nerve plexus decreases in insects poisoned by "Folidol-E 605". The same *Dendrolimus* caterpillars were used as test objects, in which the water content of the blood had been examined. The ganglion chain was dissected out after the poison had been allowed to act for 48 hours. The caterpillars were not immersed in a liquid to facilitate dissection. The ganglion chain was wound around a glass needle of known weight, and its fresh weight was determined immediately, care being taken to prevent premature drying. The

drying process was carried out over concentrated, pure sulphuric acid in vacuum.

The result revealed that on the average the ganglion chain of healthy *Dendrolimus* caterpillars contains 74.9 per cent by weight of water, whereas after poison has been effective for 48 hours the ganglion chain only contains 58 per cent by weight of water. From this, it is concluded that before all manifestation of life comes to an end, water is given off from the nerve plexus into the body-cavity and probably from other tissues, too — in caterpillars poisoned by "Folidol-E 605". This serves to strengthen the assumption that the composition of the blood is not changed solely by abnormal gland activity.

A change in the water content of the stomach ganglion chain appears to have been examined only once to date. Tobias, Kollros and Savit (1946) investigated this condition in *Periplaneta americana* poisoned by DDT but did not discover any significant change. This may probably be due to the method of dissection used. The ganglion chains they examined were placed in an eserinated salt solution from which the nerve plexus probably absorbed water again. It may have been possible of course that for these objects poisoned by DDT, there was actually no determinable difference in the water content as compared with the standard, since no histological changes in the nerve plexus were discovered following the application of poison.

Change in the concentration of hydrogen ions in the blood and in the contents of the intestine

In view of the displacement of large parts of the body-cavity fluid into the digestive tract, it would appear advisable to make a close study of the hydrogen ion concentration of both the blood and the mid-intestinal contents. Considering the excellent buffering property of the blood (Becker, 1925; Craig and Clark, 1938; Staudenmayer and Stellwaag, 1939) and the no less excellent buffering property of the intestinal contents (Staudenmayer and Stellwaag, 1939), a change of the ion composition will hardly be perceptible. A correspondingly sensitive measuring instrument is, however, of little use when the variation of the measuring results from insect to insect is so large that a significant mean cannot be obtained within a relatively short time from a number of individual results. L. Becker (1952) established in his studies of various *Lepidoptera* caterpillars, that the hydrogen ion concentration of the blood remained very constant provided the insects were of the same age and originated from the same milieu. Trappmann and Nitsche (1939) made the same finding in respect of the acidity of the intestinal contents. The silkworm caterpillars proved to be most suitable for the following studies. So that the intestinal contents would, as far as possible, be free from fodder when examined, the caterpillars were not given any fresh mulberry branches for three days. It should, however, not be overlooked that the pH of the intestinal contents is dependent upon fodder. In starved insects, the pH increases noticeably, and its buffering power, namely towards the acid side, becomes greater (Staudenmayer and Stellwaag, 1939).

An examination of the hydrogen ion concentration of the blood and the mid-intestine of untreated and "Folidol-E 605"-poisoned caterpillars of the 5th stage of *Bombyx mori* L., gave the following results:

Blood							
U	B ₁	B ₂	B ₃	B ₅	B ₆	B ₁₂	for ligated B ₁₅
6.82	6.80	6.95	6.95	7.05	7.03	7.05	6.99
6.87	6.75	7.05	7.90	6.95	6.93	6.00	7.02
6.80	6.73	7.00	7.00	7.00	6.93	7.15	6.96
6.70	6.77	6.98	7.10	7.03	7.12	7.10	
6.83	6.83	7.01	7.00	6.98	7.09	7.25	
6.80	6.77	6.98	6.99	7.00	7.01	7.11	

Mid-intestine							
U	B ₁	B ₂	B ₃	B ₅	B ₆	B ₁₂	for ligated B ₁₅
9.5	9.7	9.8	8.6	8.5	8.75	7.65	8.55
9.9	9.65	9.65	8.8	8.7	8.6	7.6	8.6
9.9	9.8	9.15	8.9	8.4	8.05	7.6	8.5
9.6	9.85	9.5	8.7	8.95	8.3	7.65	8.6
9.8	9.75	9.3	8.65	8.2	8.25	7.45	
9.7	9.7	9.4	8.7	8.8	8.1	7.3	
	9.75	9.15	8.8	8.65	8.4	7.8	
				8.55	8.15	7.75	
9.68	9.74	9.40	8.73	8.59	8.32	7.60	

The results given in the preceding table show that the pH values of the blood in silkworm caterpillars change very little following poisoning by "Folidol-E 605". But in view of the high buffering power of the blood, the slight change in the pH is nevertheless most worthy of consideration. It would appear that the hydrogen ion concentration has increased slightly in the excitation phase (B₁). During the first four hours the insects are lying on their back (B₂—B₆), there seems to be no definite change in the pH value. In this period, however, the hydrogen ion concentration is significantly lower than in the check insects (U), and has decreased to an even lower level twelve hours after the start of the test (B₁₂).

The pH values for the intestinal contents are approximately the reverse of those obtained for the blood. They decrease — the reason being that in comparison with the blood, the buffer capacity is smaller (Craig and Clark, 1938) — at a greater rate than they rise in the blood. During the excitation stage (B₁), the tendency to decrease appears to be arrested temporarily. It cannot be determined with certainty until after the insects have been lying on their backs for one hour (B₃). From then onwards, the pH continues to sink slowly. The apparent slight increase in the excitation phase would indicate that there is an increased expulsion of the strongly alkaline secretion in the mid-intestinal epithelium. The fact that the hydrogen-ion concentration does not increase to a greater degree until the insects are lying on their backs may be regarded as an outcome of the supply of saliva. The saliva which has a pH of 6.8 (Staudenmayer, 1940) that is considerably lower than the pH of the mid-intestinal contents, first accumulates in the fore-intestine before it can influence the acidity of the mid-intestinal contents after passing into the mesenteron.

The influence which the saliva has upon the change in the hydrogen ion concentration is also reflected in the pH values obtained for ligated caterpillars in which saliva is not able to pass into the intestine. In these caterpillars

(ligated B_{15}), both the intestinal contents and the blood took fifteen hours after the application of poison to attain pH values equal to those recorded for non-ligated caterpillars after only five hours. It is evident from this that the change in the pH values is closely linked with the disturbed balance following an exchange of substance due to secretion and resorption of the mid-intestinal epithelium.

Several studies have been conducted to establish whether other contact poisons change the pH values of the blood. Klinger (1936) failed to discover any considerable change after poisoning the test objects with Pyrethrum. Buck and Keister (1949), and O. Böhm (1951) were not able to establish any change, either, following poisoning by DDT. Wiesmann and Kocher (1951), however, found that the blood of *Blatta orientalis* became definitely acid after the oriental cockroach had been poisoned with an insecticide of the urethan series.

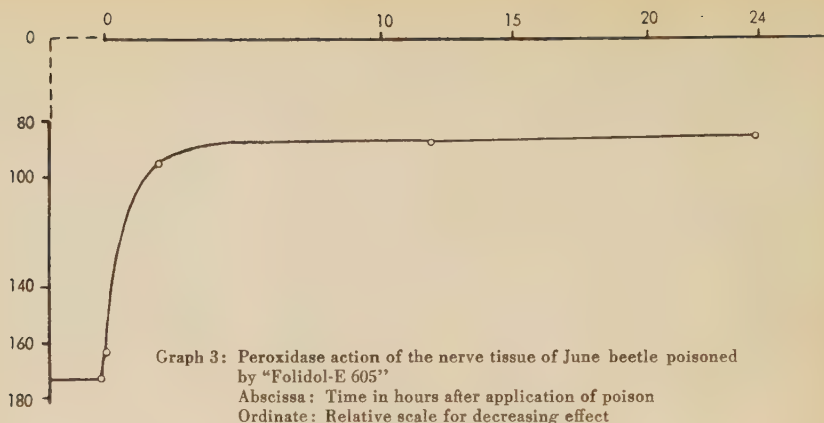
Change of enzyme actions

The considerable changes in the quantity and the composition of the blood must eventually have an influence upon the physiological-chemical processes. This point will be examined by means of several examples. It is intended to establish whether, under constant experimental conditions, there is any change in the peroxidase, the dehydrase and the catalase action of the blood. As the vital conditions of the organs circulated by haemolymph are changed along with the blood, the examination of the same enzyme actions will also be carried out with muscular and nerve tissue.

Method: It is not really possible to test all three kinds of tissue in only one insect species. The blood was taken from silkworm caterpillars in their 5th stage; June beetles were used for an examination of the thoracoabdominal ganglion masses and the cerebral ganglion, from which the pigmented eye parts had been removed; the required muscular tissue was taken from the thorax of the same beetle. It was dissected dry without immersion liquid. Tissue which had been in contact with intestinal contents, was not used. Particular care was taken that the obtained blood was not contaminated with secretion from the silk-producing glands. This secretion causes a vigorous release of the oxygen out of H_2O_2 .

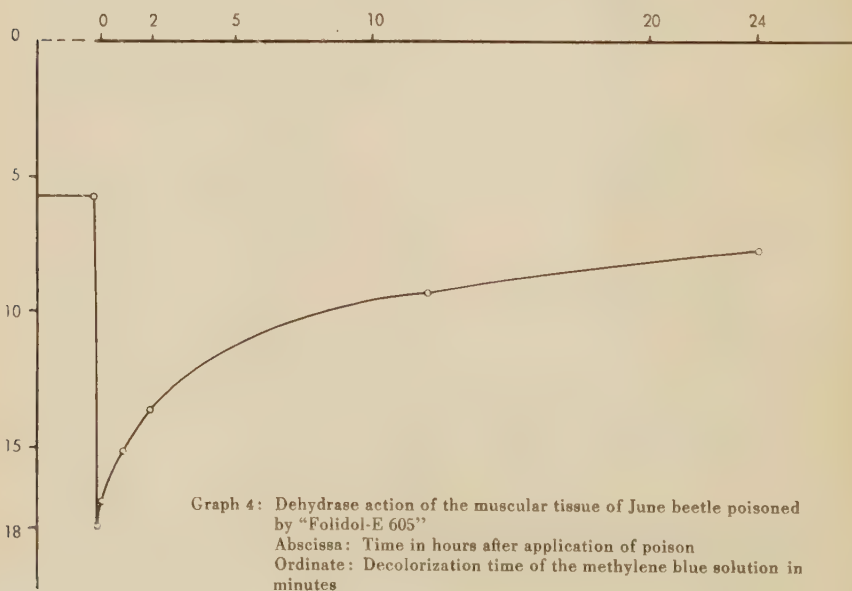
Details of the proportions determined are given in the following table:

		Blood	Muscles	Nerves
Peroxidase:		0.5 cc	15 mg	7 mg
	Aq. dest.	3 ccs	3 ccs	3 ccs
	Guaiacol 0.1 %	1 cc	1 cc	1 cc
	H_2O_2 0.1 %	1 cc	1 cc	1 cc
Dehydrase:		Blood	Muscles	Nerves
		0.2 cc	15 mg	7 mg
	Phosphate buffer pH 7	5 ccs	2.5 ccs	2.5 ccs
	Methylene blue 1:5000	0.02 cc	0.02 cc	0.02 cc
Catalase:		Blood	Muscles	Nerves
		0.2 cc	7 mg	7 mg
	Phosphate buffer pH 7	5 ccs	10 ccs	10 ccs
	H_2O_2 0.1 %	5 ccs	5 ccs	5 ccs



Result: A peroxidase action of the blood was not established either for untreated or for poisoned caterpillars. This conforms with the data given by v. Tschermack (1924), according to which peroxidases are present in all tissues, with the exception of the tissue fluids and especially the blood.

In the muscular pulp, only a very weak peroxidase action was established after 45 minutes for the excitation stage and at the beginning of the back position. It was not until 5 to 8 hours had elapsed that a very slight reaction was detected for all the other stages of poisoning and for the check.

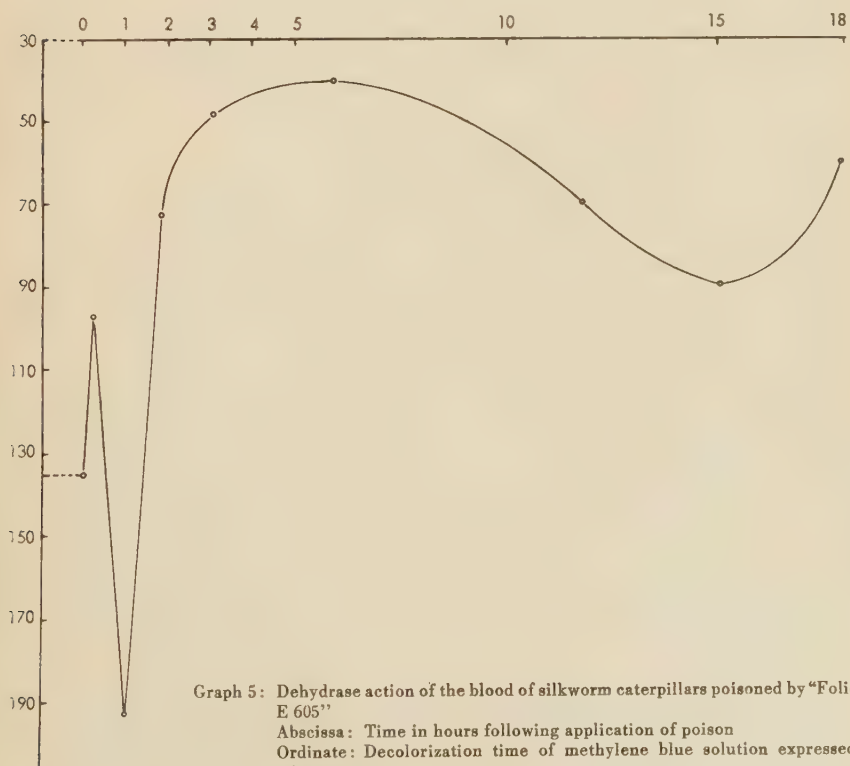


Perhaps this lack of effect is due to an inequ岸 reduction of the tissue to small pieces.

The peroxidase effect of the nerve tissue (Graph 3), however, increases greatly up to the stage when the insect is first lying on its back, and from then onwards only increases slightly, if at all.

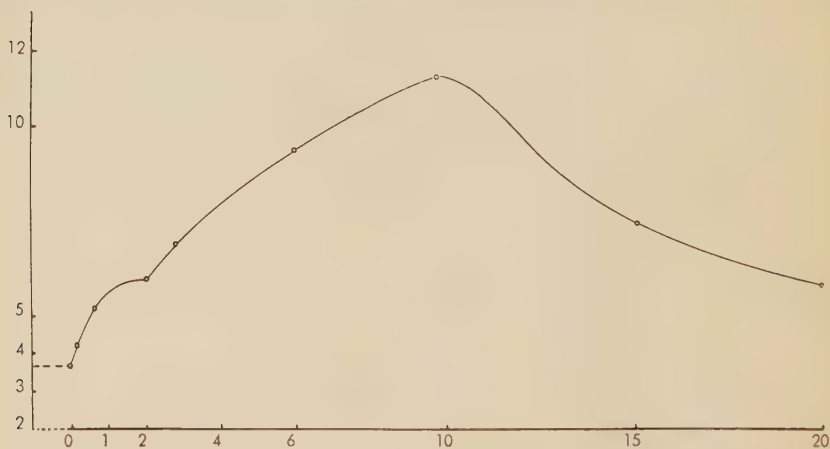
The decolorization times registered in the examination of the dehydrase action of the nerve tissue, are so long and differ so slightly over the entire period of poisoning that they can hardly be attributed to the action of the dehydrase but rather to that of the hydrogen sulphide, which can be clearly perceived at the end of the test.

For the dehydrase action of the muscular tissue (Graph 4), however, the decolorization times from the excitation stage until the initial stage of knock-down, at first give a somewhat steeper curve which continues to rise more gradually during the course of the poisoning process. Particularly noticeable is the great drop in effect during the first five minutes after the application of poison. Considering that no substrate was added to the preparation, this phenomenon was interpreted as a rapid consumption of substrate in the organism immediately after the application of poison. A dehydrase-inhibitor effect of "Folidol-E 605" (Michaelis, Arango and Gerard,



1949) most probably does not exist particularly as it can only be attained with poison concentrations which cannot occur in the insect organism (Demuth and Lendle, 1951).

The dehydrase action of the blood (Graph 5) is very similar to that of the muscular tissue. The great drop in the excitation stage is preceded by a short increase of the dehydrase effect which has been interpreted as an indication of a rapid release of the substrate immediately after the application of poison. The same is probably the case in the muscular tissue where, however, it is difficult to establish it because in *Melolontha* the poisoning process gets under way more speedily than in *Bombyx*, and the removal of the muscles takes longer than the blood. The further course of the dehydrase action until six hours after poisoning might perhaps be explained by a thickening of the blood and an increasing enzyme concentration. After the poison has been active for 12 hours, the tissue in silkworm caterpillars begins to disintegrate.



Graph 6: Catalase action of the blood of silkworm caterpillars poisoned by "Folidol-E 605"
 Abscissa: Time in hours after application of poison
 Ordinate: O₂ in ccs set free after 30 minutes

The catalase is present in the blood just as in all tissue (Wigglesworth, 1951). Considering that the oxygen uptake increases following poisoning by "Folidol-E 605", the point raised by Zieger (1951) that there is an intimate relation to the intensity of respiration, is of particular interest. Furthermore, the catalase activity is influenced by the consumption of nutrition (Burg and Leichsenring, 1921), which in view of the unnatural filling of the digestive tract, is most noteworthy. The rate at which H₂O₂ splits up is proportional to the concentration of the catalase (George, 1949); it is reversibly inhibited by high peroxide concentrations (George, 1949; Lück, 1953).

The catalase action of the blood (Graph 6) increases at a high rate after the application of poison until the excitation phase, although it certainly does not increase on the same scale at the time when the movements are more vigorous. The almost linear rise of the curve for the catalase action from the

time when the insects are first lying on their backs until the commencement of relaxation is in all probability an outcome of the increasing rate at which the blood thickens.

The catalase action of the muscular tissue remains unchanged for 12 hours after the application of poison. The stronger catalase action 24 hours after poisoning is perhaps an indication of the disintegration of muscular tissue.

The catalase action of the nerve tissue surprisingly increases during the excitation phase, and decreases gradually in the following period.

The most important results of this enzyme examination are that firstly the prerequisites for physiological-chemical processes are changed as the poisoning process becomes intensified. It is not completely unfounded to attribute the changes, in several cases, at least to an increasing enzyme concentration.

Secondly, it is established that the enzyme actions of certain tissues are intensified very soon or immediately after the application of poison.

On the basis of these studies, it cannot be stated with certainty whether enzymes *in vivo* also act more strongly following the application of poison because in these studies it was not the enzymes themselves but enzyme actions which were examined, and moreover in tissues which, removed from their association, were placed in a milieu which by no means corresponds to the conditions of life, and which is most certainly not comparable in every respect with the conditions of function changed by the application of poison. The conditions for the enzyme actions following poisoning *in vivo* are most certainly quite different from those prevailing here *in vitro*.

The cause of death

Discussion

Several symptoms of poisoning, namely atrophy of the haemolymph, greater gland secretion, bigger loss of weight and water, and increased metabolism, have been observed repeatedly. In explanation of the correlation between these symptoms it was assumed that as a result of the overstrong muscle activity which is stimulated somehow, the process of metabolism is intensified leading to a loss of weight particularly due to increased discharge of water following intensified respiration. An interpretation of this kind appears most plausible, and is strongly supported by the excessive state of excitation among poisoned insects. A critical study of published observations and results, however, shows that these cannot always be brought into harmony with such an explanation. The contradictions lose weight when the process of action as demonstrated in this paper in respect of "Folidol-E 605" is assumed to be the same also for the other lipophile insecticides, namely that as the result of an extraordinarily strong gland activity the body-cavity fluid disappears and under certain conditions is partly secreted from the body, that the process of metabolism is intensified in order to balance the resultant deficit of water, and that as a result of the increased rate of metabolism a more susceptible reaction and a persistent activity of the muscles will be rendered possible.

Wiesmann (1949) reports on a strong secretion of the poison and cutaneous glands following action by DDT in *Hoplocampa testudinea* and *Thaumato-poea processionea*. He also mentions the accumulation of this poison in the

Malpighian tubes. Bott (1948) described the secretion of fluid in several outbursts on the tarsi of amputated fly legs caused by a commercial product containing DFDT. This symptom also follows the application of "Folidol-E 605", and is easy to check and to confirm. For this purpose, it is advisable to mix this poison with the emulsifier used in commercial preparation in a ratio of 1:4; as a result, any required concentrations of poison can be prepared with water, and the mixture of poison, emulsifier and water does not become turbid. When a little India ink is added to this mixture, and a fly's leg (the wound is carefully sealed with paraffin) is placed in the liquid, it will be seen how the suspended particles are centrifuged away from the plantar surface particularly in the region of the first tarsal joints. The accumulation of haemocytes (Pistor, 1954) is easily explained by the abnormal extraction of fluid from the blood. At this stage, attention is again drawn to the findings obtained in the studies conducted by Hopp on the glands of body lice which had been poisoned by "Matacid", DDT or HCC. These findings conformed with the changes brought about by "Folidol-E 605".

Tobias, Kollros and Savit (1946) gained the impression that *Periplaneta* poisoned by DDT contain less fluid than healthy insects of this species, and that the fat body becomes smaller. Tobias, Merrill and Savit (cited after Tobias, Kollros and Savit, 1946) found, however, that the percentage of water content had remained unchanged; they did not check the body-cavity fluid but the water content of the whole insect including the intestinal contents. The decrease in the quantity of body fluid was correctly observed; the unchanged water content of the whole insect shows that a part of the blood plasma is displaced in the body. The atrophy of haemolymph which is easy to establish following poisoning of insects rich in blood, with rapidly and strongly acting insecticides like DDT, DFDT, HCC, Pyrethrum and the many different phosphoric esters, is stressed by Roan et al. (1950) who followed the change in the concentration of thoracically applied TEPP in the blood of *Periplaneta americana*. Chamberlain and Hoskins (1951) observed the same symptom following the action of "Folidol-E 605" on the same insects during the course of enzyme studies. Fritsch (1952) describes the expulsion of fluid out of the mouth and the reduction of the quantity of blood observed during his studies on the effect of the contact poisons DDT, HCC and "Folidol-E 605" after application to *Dytiscus marginalis*, *Pyrrhocoris apterus* and *Gryllus domesticus*, and speaks of an "unrestrained diuresis".

There is no mention in any of the literature of a displacement of fluid, although reference is made to an accumulation of poison in the digestive tract, particularly in the fore-intestine. Statements made by Roan et al. (1950) and by Lockau and Lüdicke (1952) to this effect, have already been quoted. The particularly high percentage of radioactive phosphorus in proportion to the dry weight, which Lockau and Lüdicke discovered in the thorax, is perhaps to be attributed to the accumulation of this element in the salivary glands which remained in the removal of the intestine. Glauco (1951) reports on observations made on non-ligated and ligated silkworm caterpillars poisoned by DDT. These observations fully conform with the symptoms established in silkworm caterpillars poisoned with "Folidol-E 605". He mentions the expulsion of large quantities of fluid out of the mouth, moreover that defaecation is normal, that the haemolymph disappears completely and that the caterpillars become smaller and dry up during the state of sickness continuing over a

period of several days. The last symptom is also observed in large *Dendrolimus* caterpillars which likewise are sick for several days after being poisoned with "Folidol-E 605". Glaucó gives particular attention to the shortening of silkworm caterpillars poisoned by DDT. He believes that it is nervously controlled. By applying a ligature between the 2nd and 3rd abdominal segments, he attempted to discover the nervous centre responsible for this. He observed on caterpillars ligated in this manner and treated with DDT, that the abdominal section no longer made the convulsive movements normally so typical, but that it remained rigid. From this, it is concluded that the nervous centre responsible for this, is located in the front half on the body, probably in the cerebral ganglion. Glaucó also mentions that the poisoned caterpillar is rendered incapable of contracting its body due to the ligature, and that it is not mummified but disintegrates. It is concluded from this that the ability to contract the body is only possible when the link between the centrally located synapses is uninjured. These explanations of Glaucó, even if they are correct, do not fully clarify the absence of body contraction and convulsions. Both convulsions and contraction of ligated caterpillars poisoned with DDT are prevented purely mechanically by the intestine due to its excessive filling and its stabilizing axis. Due to the ligation, the haemolymph present in the abdominal section is no longer accessible to the glands of the front section, and is completely concentrated in the intestine. This large quantity of accumulated fluid is opposed to the drying up of the dying tissue. In non-ligated caterpillars poisoned by DDT, however, the "mummification" keeps pace with the gradual death of the tissue, following the expulsion of a large part of the body fluid. Due to the presence of the ligature between the 2nd and 3rd abdominal segments, the start of the mid-intestine lies in the front half of the ligated section. In the illustrations of Glaucó, the very strong flatulence of DDT-poisoned caterpillars is clearly perceptible immediately before the ligature. This shows that large quantities of fluid are accumulated also in that section of the mid-intestine located before the ligature. It can also be seen from the illustrations that caterpillars which are not ligated until after the poison has been effective for one hour, have already become smaller and show less flatulence. In fact, they have already given off a large quantity of fluid before the application of the ligature. No matter how wrong Glaucó's explanations may be, his ligation tests show unintentionally and clearly that also as a result of the action of DDT, the fluid is extracted from the body-cavity and shifted to the digestive tract. An excessive flatulence of the intestine is also described by Wiesmann (1949) following application of DDT, and by Wiesmann, Gasser and Grob (1951) and Wiesmann and Kocher (1951) following poisoning with urethanes. They attribute this increase in volume to an accumulation of air which has been swallowed during the constant activity of organs consuming nutrition. The air uptake has most certainly been observed correctly. In *Musca domestica*, for example, the air bubbles can be seen through the integument circulating in the intestine. These bubbles appear to be all the more numerous, the more weakly and more slowly the poisoning process continues. They may sometimes be observed following application of "Folidol-E 605" in the intestine of the large *Dendrolimus* caterpillars which are less susceptible to poison. The swallowing of air is probably associated with a gradual and slight elimination of secretion. The insects which have become sensitive to reaction set their nutrition-consuming organs in

action when minute quantities of fluid are offered to the mouth, and because the quantity of "nutrition" is so small the swallowing and sucking action is so powerful that air may also be taken in. This is less possible, the more powerfully the insect secretes and the larger the quantity offered to the mouth. The air uptake is stimulated when the fluid offered to the mouth, particularly if and because it is so little, can be made frothy by the action of the mouth parts; the sucking-up process is similar to that when an attempt is made to draw up the very last drop of a drink with a straw. The air uptake may, therefore, be regarded as an indication of a weak poison action. It may be explained in conjunction with the displacement of fluid in the digestive tract.

It has been proved that the action of numerous insecticides causes an increase in the process of metabolism (Buck and Keister, 1949; Lord, 1949 and 1950; David, 1946; Wiesmann and Kocher, 1951). It has been established that several poisons, usually classified as contact insecticides, do not cause — or not necessarily — an increase in respiration despite their lethal action. These cases will be dealt with later.

Buck and Keister studied the effect of DDT on metabolism in *Phormia regina* Meig. The decrease in the weight of poisoned insects is increased two-fold, and continues more or less linearly for 10 hours after the application of poison. The intensity of metabolism rises linearly to approximately four times the normal value within two hours, remains at about this level for a further five hours, and then sinks gradually. The change in the intensity of breathing, particularly during the first two hours after poisoning, does not conform well with the linear decrease in weight. The authors had narcotized the flies with CO₂ before poisoning them with DDT in order to be able to weigh them better. The flies consequently spit out a drop of fluid so that they lost 8 % of their weight. When allowance is made for this loss of weight shortly before the actual start of the test — not caused here by DDT but by another poison — then the course of the changed intensity of respiration conforms really well with the course of the weight loss process. The change of metabolism, however, does not occur until the weight has decreased considerably, which is most worthy of note. This coincides with the corresponding findings on the action of "Folidol-E 605". Buck and Keister, too, cannot find any real link between the change of metabolism and the changed motor sphere. The intensity of respiration increases at a higher rate than that of movement, so that it is, therefore, ahead of the worsening state of excitation. Moreover, it is unlikely that a respiration process intensified on the average to three times the normal value over a period of six hours while the insects are lying on their backs, merely serves to meet the O₂ requirements which have arisen beforehand in 1½ hours due to the large consumption of energy in the excitation phase. Just as following poisoning by "Folidol-E 605", the metabolism probably serves here as well, to cover the loss of water, to facilitate the power of reaction and to provide the sources of energy with substance for rapid and vigorous movement. By making a comparison of the changes in weight and water content between check insects and poisoned flies, both at very high relative humidity and in a very dry atmosphere, Buck and Keister arrive at the result that neither the loss of water nor an exhaustion of the reserves of metabolism can be the primary or sole cause of death. However, the water content of the whole of the flies was determined, and no check was made to establish whether the water is present also in the vital distribution. David (1946)

also contented himself with the determination of the water content in the whole insect. After being poisoned with DDT, the larvae of *Popillio japonica* underwent a weight reduction of about 33 % due to intensive metabolism, whereas the loss of weight for the imagoes was only about 10 %. The percentage content of water in the imagoes decreases somewhat but as it remains unchanged in the larvae, the loss of water cannot be considered as a possible cause of death. David regards the toxic effect of DDT as amounting to a rapid mobilization and exhaustion of the reserves of carbohydrate. Wiesmann and Kocher (1951) point out that Pyrolan, a urethan, has the effect of causing increased metabolism and loss of weight in *M. domestica*. However, a comparison of the corresponding curves shows that the high loss of weight is not due solely to the discharge of water caused by intensified respiration. 45 minutes after poisoning, the CO₂ discharge is already below normal, whereas the loss of weight continues to increase for 18 hours. When respiration is below normal, the extent of the loss of weight can hardly be attributed to evaporation of water even when the stigmata are constantly open. Wiesmann, Gasser and Grob (1951) assume the cause of death to be exhaustion, combined with auto-intoxication (Pulver and Domenjoz, 1951).

Lord (1949, 1950) compares the many different insecticides for their effect in causing an increased rate of metabolism. Most insecticides bring about an intensification of the O₂ uptake after a varying length of time which for them is more or less characteristic. These include dinitro-o-cresol (DNOC), "Folidol-E 605", hexaethyl tetraphosphate (HETP), the γ -isomer of hexachlorocyclohexane (HCC), DDT, Chlordane, Toxaphene, Pyrethrin (mixture containing Pyrethrum I and II), but not Rotenon, Lethan B 71, Lauryl-thiocyanate and, at low concentrations, HCC and Pyrethrin. Wherever these poisons intensify metabolism, their respiration curves are very similar to each other. An exception is the curve of dinitro-o-cresol. The toxic effect of this particular substance is based on its caustifying and protein-precipitating property, so that consequently it is certainly different from that of the lipophile insecticides. An application of DNOC gives an O₂ rate ten times greater than normal. An intensification of respiration to this extent is not effected by any of the lipophile contact poisons which, at the most, give an increased intensity of respiration only four to five times greater than normal. This apparently does not only apply to the red flour beetle, *Tribolium castaneum* Hbst., which Lord used as his test insect, but also to all other insects which were used for respiration studies such as *Phormia regina*, *Musca domestica*, *Periplaneta americana*, *Popillia japonica*, *Leptinotarsa decemlineata*. This is most surprising if it is intended to attribute the increased rate of metabolism only to the very vigorous muscle activity. An explanation for this apparent hindrance and restriction of the uptake of oxygen in the case of all lipophile contact insecticides having an effect that raises the rate of metabolism, is easily found when one recalls to mind the mechanism of respiration as Wigglesworth has described it for the tracheae. It will then be clear that when there is an insufficient quantity of blood, or almost none at all, the osmotic pressure in the cells and tissues cannot be reduced any further by the removal of products of metabolism. Therefore, no more fluid can be passed into the tracheoles because it is retained in the cells by the undiminished suction power. It is the displacement of the haemolymph into the digestive tract which, therefore, hinders and restricts the uptake of oxygen in that the increased and undiminished osmotic pressure in the cells no longer permits the discharge of liquid

into the tracheoles. Accordingly, ventilation of the tracheae, brought about by the periodical change in the quantity of fluid in the tracheoles, does not take place, and the tissues only have available the oxygen which, due to diffusion in the tracheae and without movement of a fluid in which dissolution occurs, can penetrate to them through the tracheal walls.

Several symptoms which otherwise could only be interpreted with difficulty, can easily be explained by the correlations which exist between the atrophy of the haemolymph, the increase in the rate of metabolism, and the restriction of the uptake of oxygen. When fluid is extracted from the body-cavity at only a slow rate, the disappearing fluid will be replaced as the result of a somewhat intensified process of metabolism. This higher rate of metabolism then continues until the toxic substance has been rendered ineffective in the body by excretion, intercalation or chemical change, and the insect then recovers from its attack of poisoning; or, alternatively, the production of water of metabolism continues until the reserves of metabolism are completely used up, and the insect then dies of exhaustion. If, however, the metabolism has to be increased by more than three times the normal rate, the haemolymph disappears more quickly than the water of metabolism can be supplemented, and the insect dies, not of exhaustion but due to the lack of blood as a substance facilitating the harmonious team-work of the different organs. The fact that it is not the exhaustion of the reserves of metabolism which leads to death in such a case, may also be clearly recognized from the respiration curves in Lord's (1950) paper. It is illustrated that following poisoning with DNOC, far more oxygen is taken than during the course of poisoning caused by any other lipophile insecticide; in the case of the latter, therefore, it is not possible for all the reserve substance to be used up. An interesting experiment was conducted by Buck and Keister. In one instance, they allowed flies to move about for 10 minutes on a DDT-treated base, and in the other case for 30 minutes. The flies which had taken less poison, increased their rate of metabolism to a greater extent than the flies which had taken the larger quantity of poison. In the latter, the haemolymph diminished more rapidly due to the uptake of the larger quantity of poison, and therefore the uptake of oxygen was hindered earlier and more strongly than in the former. Apparently the tolerance limit for the rate of water extraction was not fully reached in the flies which had taken less poison, and the respiration had not been completely hindered, or not at all.

From the results of past studies, it can be concluded that atrophy of the body-cavity fluid due to excessive gland activity, is to be regarded as the cause of death following action by "Folidol-E 605". The consequences of fluid atrophy are increased metabolism to balance the water deficit, greater tendency to react and a larger motor sphere — both made possible by the increased rate of metabolism — and finally hindrance and restriction of the uptake of oxygen because with an inadequate quantity of blood the exchange and translocation of products of metabolism can no longer take place, and consequently the undiminished osmotic pressure in the cells and tissues renders impossible ventilation of the tracheae brought about by a change in the quantity of fluid in the tracheoles.

According to the published results of investigations into the action of other contact poisons, it is highly probable that these poisons unfold their fatal effect in the same manner.

The question of the site of attack

I. Histological examination of the ganglion cells

The first fatally effective change established in these studies is the hyperfunction of the gland tissue. Thus the question is also raised as to what causes the intensified function of these organs. Normally the labial gland begins to function on nervous impulse. The mid-intestinal epithelium, and its activity, also in insects, is in all probability controlled humorally (Day and Powning, 1949). An increased gland activity is possible in many ways. It would appear advisable to examine whether the poison stimulates the activity of the nerves, whether it acts in the substance released at the nerve ending and also influences this substance's mechanism of action, and finally whether there is a facilitation of function due to a change in the effectors.

The nerve cells and tissues have frequently been examined under the assumption that the motor sphere is abnormal. Investigations were made to establish whether the nerves, in particular, are harmed by the poisons and whether characteristics are manifest in the injury, which are specific for the different poisons (Klinger, 1936; numerous papers of Hartzell and different co-workers 1932, 1934b, 1934c, 1942, 1944, 1946; Wigglesworth, 1941; Witt, 1947; Böhm, 1951; Hopp, 1953; Lüers et al., 1953, 1954). Histological changes were established for all contact poisons examined. The changes, however, are of a completely general nature. Specific characteristics cannot be quoted for the different poisons, and the changes do not permit a definite conclusion to be drawn as to their causes. Richards and Cutkomp (1945) emphasized this in their paper.

Despite the widespread assumption that "Folidol-E 605" is a nerve poison which acts centrally, only two papers are available reporting on histological studies conducted to investigate this question (Th. Lüers, H. Köpf and H. Lüers, 1953; K. Pistor, 1954). Th. Lüers et al. studied the question of the morphological substrate of "Folidol-E 605" poisoning in the central nervous system of *Drosophila* and also of *Musca*. An examination was made of the changes in the final stage. At this phase in the poisoning process, it was established that among the large nerve-cells, there were a number which were unchanged and others showing a high degree of swelling and extensive chromatolysis. The nuclei in these cells are also swelled. No changes could be established in the nucleoli. The medium-sized and small cells had shrunk in certain cases, and together with their nuclei were exceedingly stainable. The authors arrive at the conclusion that there may be various causes for such changes, which do not permit any conclusions to be drawn as to a specific effect of "Folidol-E 605". On the whole, Pistor's findings are the same for *Calliphora*.

Even though no specific changes can be established in the ganglia and the nerve-cells, this does not preclude the possibility that the nerve tissue is harmed very soon after the application of poison.

It is, therefore, intended to establish when the first changes appear in the nerve-cells following the application of "Folidol-E 605", and whether the abnormal gland activity can possibly be explained by microscopic changes of the nerve-cells, which become visible at an early stage.

Technique: The ganglia of the ventral nerve cord in silkworm caterpillars poisoned by "Folidol-E 605" were examined. All preparations were pro-

duced in exactly the same manner. The ganglion cords were dissected out without an addition of liquid, and fixed in acid-free Formol. The 8 μ thick sections were stained with gallocyanin. The constancy of the method guaranteed that any differences in the colour intensity are actually caused by the object.

Result: Only changes occurring simultaneously were established in the ganglia of one cord. They are described below by means of several illustrations.

The nerve-cells of insects are usually classified in three types. There are the large nerve-cells (NC I) with bright nuclei poor in chromatin; the cytoplasm of these nuclei is filled with a substance which can be strongly stained with certain stains (Nissl, tigroid and chromophil substance). The medium-sized (NC II) cells have a thicker nucleus which takes up the greater part of

Figs. 12 to 18: Sections of the different ganglia of the *Bombyx mori* L. caterpillar
Fix.: Formol 1:25; Stain: Galloeyanin; pH = 3.2; Sectional thickness: 8 μ

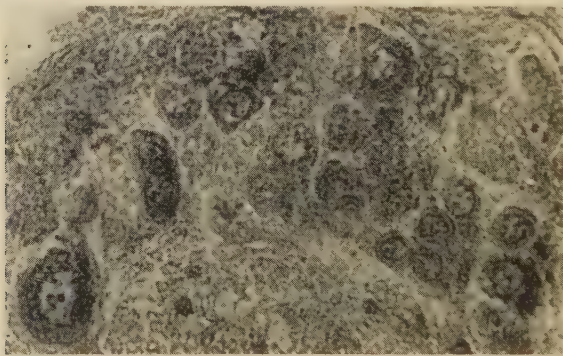


Fig. 12: 1st abdominal ganglion of a normal caterpillar. Magnification : 430

the cell body; the narrow plasma border is filled with chromophil substance here, too. The small cells (NC III) consist of a dense nucleus with only a very narrow plasma border containing no Nissl substance. The small cells (NC III) only occur in the cerebral ganglion. The large nerve-cells in the individual ganglia are chiefly located close to the connectives. The medium-sized nerve-cells form the main mass of the nerve-cell layer. Whether a nerve-cell belongs to the middle-sized type or a small cell represents the large type cannot always be decided with certainty as long as they are characterized only by morphological features. The nerve-cells form a compact peripheral layer in the ganglia, which is interspersed with tracheae and tracheoles. The interior of the ganglia, the neuropile, consists of a compact mass of nerve-fibres. The two NC types of an abdominal ganglion are illustrated in Fig. 12. The cells are cut at various planes.

In poisoned caterpillars, the first changes are in the late stage of excitation, shortly before the caterpillars finally lie

on their sides. The stainability of the individual nerve-cells now differs most perceptibly. The large NC take the stain more fully than the others but no longer to an equal degree in the whole plasm region of the individual cells. The stainability in the region of the cell processes has become very much weaker (Fig. 13, top right). The NC II are only very weakly stained in groups. The nucleoli mostly stand out more fully (Fig. 13, bottom right). One cannot speak with certainty of a cell swelling among the NC I in this late stage of excitation. It appears, however, as if the nuclei of the NC II have become larger through chromatolysis.

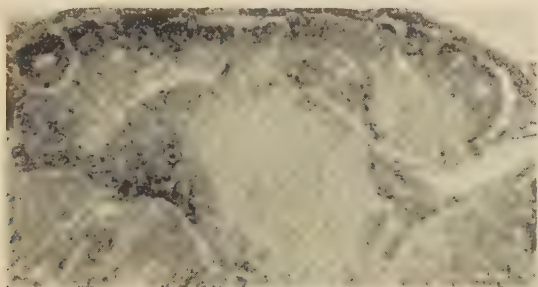


Fig. 13: Prothoracic ganglion of an "Folidol-E 605"-poisoned caterpillar in a late stage of excitation. Magnification : 340

In the early stage of the back position when the caterpillar still makes vigorous movements, the ganglion cell layer takes the stain more fully again. In certain cells, the chromatolysis is more advanced, among both the NC I (Fig. 17) and the NC II. The nuclei of the NC II, whose chromophil substance has practically disappeared, appear to have swelled most considerably. Moreover, the NC II exhibit signs of shrinkage to a varying extent. Some of

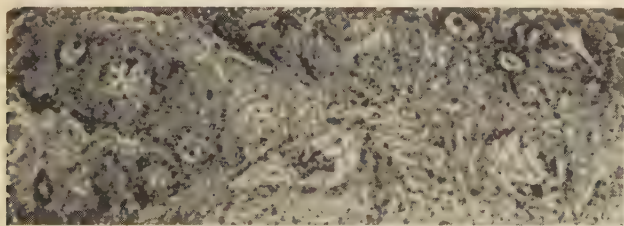


Fig. 14: 2nd thoracic ganglion of an "Folidol-E 605"-poisoned caterpillar in the early stage of the back position. Magnification : 430

the nuclei have lost their smooth surface, and have collapsed into very small structures. With the atrophy of Nissl substance, the chromatin at first assumes lumpy forms, and also appears to lessen. The nucleoli stand out more clearly in the light cell nuclei (Fig. 14). The nucleoli also appear to have become larger without losing any of their staining property. Often it is noticed that they are connected to the nucleus membrane by a fully stained cord in their

central location (Fig. 15). In shrivelled nuclei, the nucleoli have also become smaller. In several cells, the almost empty looking nucleus is bordered by a narrow, deeply stained line (Fig. 14); judging by cell images in Fig. 15, it may be assumed that this is Nissl substance which has not yet dissolved or is in the process of dissolution. With well advanced chromatolysis, this substance lies in a very thin layer on the severely swelled nuclei, and surrounds the strongly shrivelled nuclei, correspondingly dispersed.

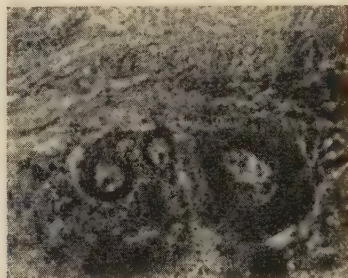


Fig. 15: 3rd thoracic ganglion of an "Folidol-E 605"-poisoned caterpillar in the early stage of the back position. Magnification : 430

It is, however, not intended to dispute that chromatin may also have a part in the coloured marking of the nucleus boundaries. A most striking feature observed after the insects have been lying on their backs for one hour is the unusually deep colour of the Nissl substance in many NC I and NC II (Fig. 16). The dark-coloured NC II are no doubt shrivelled and deformed (Fig. 16, top right). This cannot be said, however, of the likewise coloured NC I although the Nissl substance obviously no longer fills up the whole of the space it normally does. The nuclear volume of the NC I has not increased — or apparently not — although that of the NC II has become larger insofar as these cells have not already shrunk (Fig. 16). On the whole, the cell layer gives the impression of being slightly disintegrated, most particularly in the region of the shrivelled NC II; loose fibre structures can be recognized between the deeply coloured NC I.

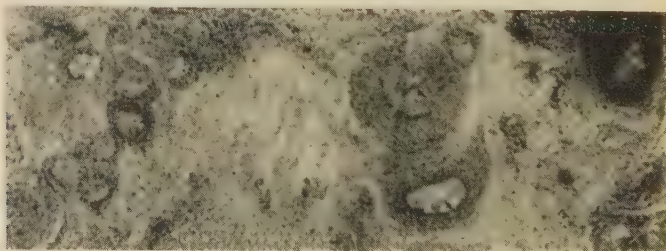


Fig. 16: 3rd thoracic ganglion of an "Folidol-E 605"-poisoned caterpillar after lying on its back for 1 hour. Magnification : 430

A tissue pattern forms in the ganglia of *Bombyx* caterpillars after "Folidol-E 605" has been effective for 12 hours, in the final stage (Fig. 17). This pattern can be well compared with that described by Lüers et al. for *Drosophila* and *Musca*. Next to non-coloured NC I with greatly swelled nuclei, partially coloured cells are to be found which together with their nuclei, have shrunk. The majority for the NC II have collapsed completely, and sometimes they can hardly be identified as cells. The cell layer merely comprises loosely formed tissue.

After the poison has been effective for 24 hours — the caterpillars have been in a relaxed state for some considerable time and are no doubt already dead — the picture of complete disintegration is more intensified (Fig. 18) but is not much different from the final stage.

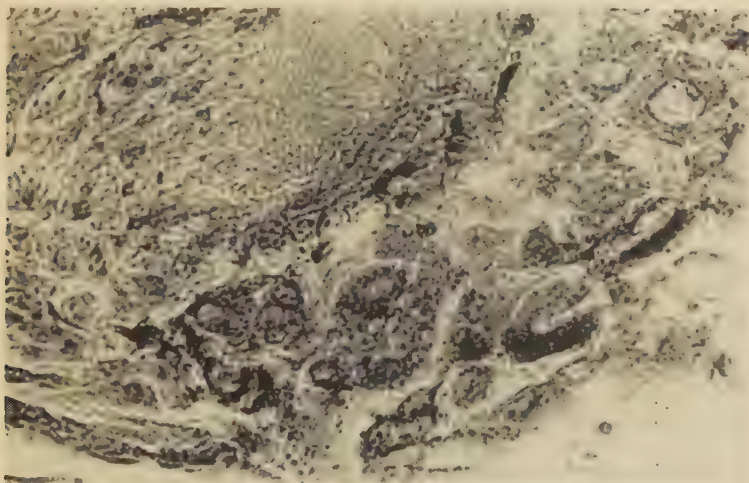


Fig. 17: Abdominal ganglion of a caterpillar after being subjected for 12 hours to poisoning by "Folidol-E 605". Magnification : 430

The most important result of this study is that the first changes in the nerve-cells of silkworm caterpillars poisoned by "Folidol-E 605", are not established until the late stage of excitation. These changes are only slight and they can hardly be termed as pathological. The poor stainability of the medium-sized neurones may probably be interpreted as meaning that their cell content has been diluted due to the uptake of fluid from the surrounding area — probably from the tracheoles of which there are very many in the nerve-cell layer of the ganglia; the nucleus and cell size of these neurones would appear not to preclude an explanation of this nature. In fact this explanation is supported by the limitation of the oxygen uptake. The changes that take place in the nerve-cells following application of "Folidol-E 605" are of the same kind already established by numerous authors following application of the different contact poisons. For this reason, reference is made to Lüers et al. (1953, 1954) and Richards and Cutkomp (1945) for an explanation of these changes.

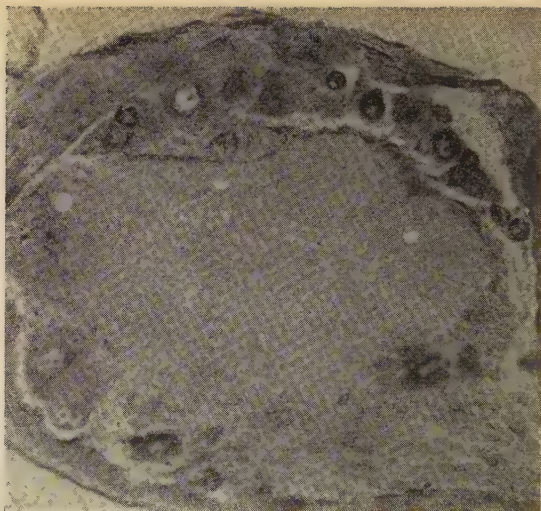


Fig. 18: 2nd thoracic ganglion of a caterpillar subjected for 24 hours to poisoning by "Folidol-E 605". Magnification : 350

II. Discussion of possible sites of attack

The changes in the cells and ganglia brought about by the action of the many different contact poisons are all of the same nature although they may differ in the degree of their development. In fact it happens that no changes are visible following application of DDT despite fatal sickness (Tobias, Kollros and Savit, 1936; Witt, 1947; Lüers et al., 1954). Even when a disease of the nerve plexus cannot be established microscopically, it does not mean that an injury to this organ by the poison is precluded.

The presence of a lipophile contact poison in the nervous system has, however, not been definitely established to date despite the employment of very fine methods of examination. Mention should be made here particularly of the studies conducted with insecticides labelled with radioactive atoms. In assessing the results obtained with these labelled insecticides, it must be borne in mind that one cannot always conclude from the presence of radioactive isotopes that molecules in which the isotopes were incorporated, are also present. Hansen, Hansen and Craig (1944) investigated the distribution in the body of *Periplaneta americana*, of a DDT-homologue of dibromodiphenyl trichloroethane prepared with ^{32}Br . They washed off by means of alcohol the poison still adhering externally 24 hours after application, and then prepared radioautograms with sections or spread-out organs. They established that "just as in other parts of the insect, the material is present in the brain and in the fasciculus of nerves". Unfortunately, this paper — it was published in 1944! — is extremely short, and contains neither detailed information nor a reproduction of a radioautogram. It is quite feasible that while the sections were being prepared and the organs spread out, blood was also distributed, which at that time had not yet been recognized as the chief distributor of poison in the body. Lindquist, Roth, Hoffmann and

Butts (1951) found only a very slight quantity of radioactive substance in the many different tissues and organs in the thoracic ganglia of DDT-resistant houseflies 24 hours after they had been treated with radioactive DDT. The quantity traced amounted to only 3 % of the poison which had penetrated into the body. This is so small as to be within the limit of error. When it is considered — and this also applies to the studies of Hansen et al. (1944) — that the ganglia not only consist of nerve substance but also contain, for example, connective tissue, tracheae and tracheole fluid, furthermore that most of the poison is quickly broken down due mainly to resistance (Ferguson and Kearns, 1949; Sternburg, Kearns and Bruce, 1950; Lindquist, Roth, Yates, Hoffmann and Butts, 1951), and that the radioactive atoms no longer belong to the DDT molecule and have possibly penetrated also into the nerve tissue in the process of general metabolism, this test result cannot be taken as supporting the presence of poison in nerves. An investigation conducted after the poison has already been effective for 24 hours will hardly provide any useful information on the site of attack of a poison which becomes strongly effective only a few minutes after it has been applied. The possibility of DDT penetrating and spreading in dead nerves (Wiesmann, 1949), and the stronger bond of the lipophile insecticide in a homogen of the nerve tissue rich in lipophiles as compared with a pulp of muscle or adipose tissue (Ball and Beck, 1951), merely show that the chemical prerequisites exist for the uptake and retention of the poison but disregard the selection powers of living cells and tissues. The delayed occurrence of poisoning symptoms following mechanical injury of nerve tissue by the removal of a section of ganglion chain or cauterization of a ganglion, cannot be taken as proof of the presence of the poison or a toxic transformation product and its translocation in nerves. This only demonstrates a hindered transmission of excitation which in its form need not be changed. In fact Ball and Beck (1951) could not establish the presence of "Folidol-E 605" in living nerve tissues either by chemical analysis or by means of biological test methods. This fully conforms with the findings of Roan, Fernando and Kearns (1950). These authors used several radioactive phosphoric esters and established, particularly with TEPP (Schradler, 1951), the distribution of ^{32}P in the different tissues and organs at various times following application of the poison. But in no case did they discover a decomposition of radioactive phosphorus atoms in the nerve tissue, which might justify the assumption that these toxic substances are taken up in the nerves. Lockau and Lüdiche (1952) observed strong decomposition of isotopes in the head of *Periplaneta americana* after being poisoned with radioactive "Folidol-E 605". They therefore believe that on the strength of this finding, they can conclude that the poison is present in the cerebral ganglion. In view of the unusually long poisoning times for the substance concentrations used, and particularly of the fact that the quantities of applied substances do not conform with the knock-down times, considerable doubt arises as to whether the substance used as poison was actually "Folidol-E 605", or "Folidol-E 605" in the given concentration. Moreover, the conclusion is by no means cogent; the head does not only contain the cerebral ganglion, and it does not constitute the main proportion of the dry weight, either. The head also contains glands, muscles and tracheae. It should also be especially remembered that the blood which is constantly becoming thicker, is pumped by the heart and the aorta into the head where, due to its viscosity, it remains in the organs located there. Conse-

quently the poison transported with the blood is accumulated in the head. The same applies to the accumulation of DDT in the head (Roux and Morrison, 1954). It may, therefore, be asserted that despite numerous and thorough investigations, the presence of contact poisons in living nerve tissue has not been completely established.

Occasionally, it has been assumed that it is not the applied poison itself but a decomposition or transformation product which acts toxically. In several cases, this assumption has already been refuted (cf. Rocka, 1950). Ball and Beck (1951) and Chamberlain and Hoskins (1952), in particular, speak of phosphoric esters having such an action. The former are forced to make this assumption because they adhere to the theory that the toxic effect starts from the nerve tissue, the latter because they regard the action of phosphoric esters as merely amounting to a blockage of the cholinesterase action, although the inhibition of the cholinesterase by "Folidol-E 605" is slower and less complete *in vitro* than *in vivo*. There is, however, no forcible reason why one should adhere strictly, to either the one or the other viewpoint.

It has sometimes been assumed that the lipophile insecticides attack the mechanism of excitation transmission from the nerve to the muscle, particularly in the ACh-ChE system (Metcalf and Kearns, 1945; Luger, Martin and Muller, 1944). This assumption has proved to be wrong in respect of DDT and HCC (Tobias, Kollros and Savit, 1946; Truhaut and Vincent, 1948; Chamberlain and Hoskins, 1951). The phosphoric esters, on the other hand, actually inhibit the ChE and cause an accumulation of the ACh in the body (Metcalf and March, 1949, 1950; Lord and Potter, 1951; Chamberlain and Hoskins, 1951; Duspiva, 1951; Brauer and Pessotti, 1949; Aldrige, 1950; Hartley and Brown, 1955). It has also been established that new insecticides belonging to the urethan group have an inhibiting effect on several esterases (Pulver and Domenjoz, 1951). According to the findings of certain investigations, it is really questionable whether the ACh and the ACh-E play the same part in insects as they are assumed to do in vertebrates (Metcalf and March, 1950; Chamberlain and Hoskins, 1951; Lord and Potter, 1951).

It is definite that in insects the neuromuscular mechanism of action is different from that in vertebrates, and their organs react to the different substances — which are capable of changing the nervous effects in vertebrates — not only quantitatively but also in a completely different manner, sometimes in just the opposite manner. In fact among the insects, the same action substances do not appear to act in the same manner on all species (Pringle and Hughes, 1948; Krijgsman and Krijgsman, 1950; Kooistra, 1950; Metcalf and March, 1950, 1953; Lord and Potter, 1951; Babers and Pratt, 1951a; Chamberlain and Hoskins, 1951; Roan and Maeda, 1954). In keeping with the incompleteness of our knowledge on the occurrence of nervous functions and on the nature and effect of nervous action substances particularly in insects, it is just as impossible to term the inhibition of an esterase action as the site of attack of a poison. Several findings in fact indicate that the inhibiting effect on the ChE produced by insecticidal phosphoric esters is only of secondary importance for the toxic effect. Metcalf and March (1949, 1950) examined the ChE-inhibition and the toxic effect of a large number of phosphoric esters. They established that on the whole, both effects are parallel. It is, however, most noteworthy that several substances apparently are an exception. Lord and

Potter point out that the toxic effect cannot be explained solely by esterase inhibition. Chamberlain and Hoskins (1951) find that in *Periplaneta* poisoned with "Folidol-E 605", the ChE is inhibited much more strongly than is possible *in vitro*, in other words that here, too, the increase of the ACh cannot be explained solely by the inhibiting effect of "Folidol-E 605" on the ChE. In their opinion, it is quite feasible that "Folidol-E 605" is transformed by certain living tissues into a stronger esterase inhibitor, perhaps into "E 600" (Schrader, 1953). Duspiva (1951) obtained similar results concerning the action of Pestox III. This unexpectedly great increase of the ACh content in living insects reminds one very much of results obtained by Kollros and Savit (1946). Accordingly, the ACh content in *Periplaneta* increases considerably also following application of DDT and HCC although these two poisons are not esterase inhibitors nor do they accelerate the synthesis of ACh. On the contrary, the bonded ester is converted into the free form chiefly in the connectives of the abdominal ganglion chain, although this does not take place until after the overstrong excitation phase, which is most remarkable. The unexpected increase of the ACh after the application of "Folidol-E 605" is probably due to the same cause as that established for the increase of this action substance following application of DDT or HCC. The increase in the rate of metabolism to balance the deficit of water, also stimulates due to the raised body temperature, the metabolism and the development of physiological processes in the ganglia and nerves. The hindrance of oxygen uptake and the quantitative and qualitative change of the blood, harm the nerve tissue which now converts the bonded ACh into the free form (Murralt, 1946). The loss of water, moreover, allows the ACh content to increase whereby the ChE may be inhibited, although not irreversibly (Augustinsson and Nachmansohn, 1949). The deficient body-cavity fluid which no longer circulates sufficiently, and the changed physiological state of the cells might unfavourably influence the ChE effective on the cell boundaries. Most particularly, the harmful effect of a high ACh-content, and thus the significance of the ChE-inhibition of the phosphoric esters for the toxic effect, appears to be in doubt as the result of more recent investigations conducted by Aldrige and Barnes (1952) and Aldrige and Davison (1952) who demonstrated that the effect of ChE-inhibition is determined by impurity of the phosphoric ester, and that pure "Folidol-E 605" can at the most be termed as a weak *in vitro*-inhibitor. Hopf (1952) did not succeed in establishing a ChE-inhibition by phosphoric esters in the large migratory locust, either. After all that has been said, the assumption appears to be well founded that also with respect to the influence upon nervous action substances, there is on principle no important difference between the effects of DDT, HCC and "Folidol-E 605". There is much to be said for the assumption that other factors play an important part in the effect of "Folidol-E 605", and the doubt expressed that the ChE-inhibiting effect of "Folidol-E 605" is the reason why poisoning sets in rapidly (Schrader, 1951).

It is clear that results of investigations in which no consideration was given to the change of the blood and the increased rate of metabolism, can hardly be taken into account in order to provide an explanation of the toxic effect, even though they may be most interesting. Above all, this includes investigations of isolated organs or organic systems, and those placed in artificial media. It has already been mentioned that they do not react to poisons in the same way as they do *in situ* (Fritsch, 1952).

From the homogeneous progress of poisoning following application of the many different lipophile contact insecticides, it is not possible to conclude that these poisons attack at different sites. As a result of the more or less similar effect, the assumption is strengthened that the organic contact poisons, particularly because of their lipophile property, cause physicochemical changes, corresponding to their affinity to the cell lipides. Such a site of attack in the system of the cell structure might provide a uniform explanation for the similar effect of these substances. It is by no means restricted solely to insects or the animal world, but is also evident in a comparable manner on plants corresponding to their respective organisation (Frohberger, 1949; Lins-kens, 1951, 1952; Tietz, 1953; Wäckers, 1955). It would thus be comprehensible why organs which do not begin to function following nervous impulses, are also affected by them. Unfortunately, we know too little about the nature and construction of cell lipides and their physiological significance in order to make any definite statements.

Summary

1. Following the application of "Folidol-E 605" as a contact poison, the glands of the digestive tract in insects are stimulated to form secretion in a most vigorous and persistent manner. The superabundant quantities of secretion formed may be discharged through the mouth opening. The nature and quantity of the discharged secretion depend upon the quantity of blood present in the different insect species; also upon the nature and function of the glands, and upon the construction features of the digestive tract.
2. Weighings of secretion discharged during the process of poisoning indicate the extent and intensity of fluid displacement in the insect organism. The unnaturally high loss of fluid comes to an end in the initial stage of the excitation phase. The excretion of solid and fluid substances during the remainder of the poisoning process is no greater than in normal insects.
3. Histological studies show that the activity of the glands increases even before any symptoms of poisoning become apparent from the behaviour of the insect.
4. In addition to the secretion of fluid, there is also an abnormal increase in the elimination of gases from poisoned insects.
The intensity of oxygen uptake is already above normal before commencement of the excitation phase.
The O_2 rate increases linearly from before the start of the excitation phase until the poisoned insect is more or less incapable of reacting.
5. From the commencement and the continuation of the intensified process of metabolism, it is obvious that the increased uptake of oxygen cannot be induced by motor hyperactivity, nor is it additionally increased by excessive muscular activity during the excitation phase. It is concluded that the intensification of metabolism is caused by a lack of fluid in the body-cavity, and that in the first place it serves to produce water of metabolism. The hyperexcitability and the motor hyperactivity following the intensification of metabolism, are automatic secondary symptoms resulting from the supernormal release of heat energy and the formation of superabundant products of metabolism.

6. As a result of the heavy secretion of fluid arising out of supernormal glandular activity, the water content of the blood is reduced and the composition of the dry substance changed.
7. The constant limitation of the O_2 rate at a maximum equalling on the average four to five times the normal value, is explained as the outcome of the pronounced change in the quantity and composition of the blood plasma and the resultant hindrance of the exchange and translocation of products of metabolism, in the sense of Wigglesworth's theory of respiration.
8. The nerve tissue of the *Dendrolimus pini* L. caterpillar, in which the process of poisoning continues over a relatively long period, is taken as an example to show that the large extraction of fluid from the haemolymph can also lead to a loss of water in other organs.
9. The hydrogen ion concentration of the haemolymph decreases and that of the mid-intestinal contents increases when the blood undergoes a change.
10. The peroxidase, dehydrase and catalase actions of the blood and the muscular and nerve tissue were studied during the process of poisoning. The manner in which the enzyme actions change, may be interpreted to a certain extent as being due to an increasing enzyme concentration.
11. The histological examination of the ganglion chain did not reveal any specific changes after poisoning with "Folidol-E 605". The first changes appear in the form of cell, nucleus and nucleolus hypertrophia, at the earliest in the late stage of excitation.
12. The atrophy of the haemolymph in the region of the organic structure resulting from the displacement of body-cavity fluid into the digestive tract, is to be regarded as the cause of death to insects following poisoning with "Folidol-E 605".
13. In the discussion, it may be pointed out that application of other lipophile contact poisons causes the same course of disease as "Folidol-E 605".
14. With respect to the site of attack, it is assumed that all organic lipophile contact insecticides act in the same manner — even though at varying strength — because they cause changes in the cell boundary layers due to, and corresponding to their affinity to the cell lipides.

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